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CYTOPLASMIC STRUCTURES IN THE MALE GERM CELLS OF RHOMALEUM MICROPTERUM BEAUV.

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In a recent paper Lewis and Robertson gave an account of certain cytoplasmic structures in the male germ cells of *Chorthippus curtipennis* Scud. as seen by the tissue culture method. It is the purpose of this paper to give somewhat similar data for the Florida lubber grasshopper, *Rhomaleum micropterum* Beauv., on the basis of both fixed and living material. My observations were made mainly in order to determine the origin and significance of the chromatoid body. They closely parallel those of the previous authors, and may perhaps clear up some doubtful points.

While studying the maturation phenomena in *Rhomaleum* in 1914 it was found that a small round densely staining body was always present in the cytoplasm of the cells of the late growth period. Further study disclosed the fact that this body appeared in the very early growth period, enlarged up to the stage of diakinesis, and at both the first and second maturation divisions passed unchanged into one of the two daughter cells. During the metamorphosis of the spermatid this body passed gradually down into the tail region of the future spermatozoon, and was eventually cast off and degenerated in the lower end of the follicles. This history is exactly parallel to that of a similar but larger body described by Dr. E. B. Wilson ('13) in the spermatogenesis of the hemipter *Pentatoma senilis*, and called by him the "chromatoid body." It is now known that a granule or granules of similar behavior are present in the developing male germ cells of the following forms: horse, pig, bull, rabbit, crayfish and

probably in the rat, mouse, and a number of Hempitera. It seems probable therefore that the appearance and elimination of such material from the spermatozoa is a fairly general phenomenon throughout the animal kingdom.

The recent study of Lewis and Robertson on the living germ cells of *Chorthippus curtipennis* showed that certain granules were present in the spermatogonia and passed on somewhat enlarged into the metamorphosing spermatids. From the fact that these granules stained with neutral red in contrast to the mitochondria they were called "neutral red granules." Up to the time their paper was published the work with *Rhomaleum* had been carried exclusively with fixed material, but in view of the similarity of behavior between these granules and the chromatoid body in the Florida grasshopper it was believed that they were of similar material and significance. Work on the living male germ cells of *Rhomaleum* during the past summer has confirmed this belief in every respect, and has made it possible to add a few facts to the data of the previous authors.

The material from which the observations were made was secured from the Supply Department at Woods Hole during the summers of 1915 and 1916. That used for permanent preparations was fixed in the usual fixatives including the modified Flemming's fluid used for the Benda mitochondrial technique. A large number of different staining methods were employed including the Auerbach mixture, the Borel stain, together with the Altmann acid fuchsin stain and the alizarin-crystal violet combination of Benda for mitochondria. The osmic mixtures were invariably the best cytoplasmic fixatives and from material so treated all of the drawings from permanent preparations were made. The methods used with the living material were for the most part those of Lewis and Robertson. Intravitam staining with janus green B and neutral red was employed, the stains being used both separately and together. The culture medium used was the modified Locke's solution of these authors made up with sea water, though some follicles were stained in Ringer's solution with fair success. The tissue cultures were only partially successful, most of the cells being abnormal after thirty-six hours. This was probably due to lack of familiarity with the

technique. Ample data for the purposes of this paper were secured, however, since cells can be secured at any desired stage without watching any particular cell for any great length of time.

Observations.

However we may interpret the behavior of the chromosomes, *Rhomaleum* corresponds in all essentials with the account of orthopteran spermatogenesis given by Davis ('08) for *Dissosteira*, with the *Hippiscus* type of the Acrididæ as described by McClung ('14) and with *Phrynotettix* (Wenrich, '16). A connected account of the stages through which the chromosomes of *Rhomaleum* pass during the growth period would therefore correspond in most respects with that given by Davis.

In the spermatogonia the cytoplasm in fixed material is comparatively clear except for a minute fibrillar network. In addition several minute but darkly staining granules can usually be seen scattered about the cytoplasm. Mitochondria are apparently never visible in the spermatogonia even when the material is fixed in the modified Flemming and stained with the so-called mitochondrial stains. In living material however the cytoplasm shows no fibrillar network, but the mitochondria appear irregularly scattered about the cytoplasm or more often in a fairly dense mass close to the nuclear wall. In the resting stages in *Rhomaleum* they are very delicate granules, staining brilliantly with janus green, and show very little tendency toward any thread-like arrangement. No dividing primary spermatogonial cell was observed. In the resting stages of the secondary spermatogonia the mitochondria apparently have a tendency to be less scattered and more densely crowded next to the nuclear wall (Fig. 1). In dividing secondary spermatogonial cells the mass of granules is arranged in irregular rows about the spindle, and fairly evenly divided without assuming any definite thread-like form. The granules visible in the fixed material can also be seen in the living cells. They are usually five or six in number, and do not stain with janus green. After being treated with neutral red for about an hour, however, they appear faintly pink. The number is often as large in the secondary as in the primary spermatogonia, and this gives some reason for supposing that

new granules of this sort are being gradually formed. While these granules cannot be followed during the spermatogonial divisions there seems little doubt that they lie inert in the cell and their distribution is merely hit or miss to one or the other of the daughter cells.

After the last spermatogonial telophase the nuclei enter on the stage shown in Fig. 2, in which the chromatin appears as a light network in prepared material. This network shortly becomes aggregated into flocculent masses corresponding roughly in number to the diploid number of the chromosomes. This "massive body" stage is beginning to appear in Fig. 3 and Fig. 4. The mitochondria in these early stages of the growth period are extremely difficult to demonstrate in fixed material—as indeed they are throughout the spermatogenesis—even by the special technique for mitochondria. At times, however, a cloudy mass can be seen forming a cap over one side of the nucleus (Fig. 2). With janus green, however, they become very prominent in the living material. The granules are larger and seemingly in greater numbers than in the spermatogonia. The details of their behavior are similar to those in *Chorthippus*, except that here again there is no tendency to assume the thread-like form.

Throughout these earlier stages of the growth period a mitosome, the remains of the last spermatogonial division, is present. Often an actual bridge between the two daughter cells persists for a short time after the division is complete. This is the condition seen in Fig. 3. The mitosome rapidly disappears after the stage shown in Fig. 3, and is never found after the "massive body" stage. At about this time there appears in about the center of the cytoplasmic mass a more or less definite sphere which is browned by osmic and only slightly stained by hæmatoxylin. At first I took this to be a mitosome, which it strongly resembles, but later it was found that a definite mitosome was present at the same time, as shown in Fig. 3. The sphere is shown with great clearness in Fig. 4, where it lies in a large vacuole—probably due to imperfect fixation—which is surrounded by masses of mitochondria. This sphere is not visible in the living cells, whether stained or not, but its presence can be inferred from the fact that in the growth period the mito-

chondria are usually in two separate masses with a clear space close to the nuclear wall between. An extreme case of this sort is shown in a cell in the bouquet stage (Fig. 6) which will be referred to later. Here it is very clear that a spherical mass must separate the mitochondria. This characteristic clumping of the mitochondria in two groups is mentioned by Lewis and Robertson, but no explanation is attempted. The behavior of this sphere shows that it is in the nature of an attraction sphere or idiozome (Meves).

Finally there are in the cytoplasm of these cells of the early growth period from three to six or more of the "neutral red granules" mentioned above. They are larger and more prominent than in the spermatogonia, and are shown stained with hæmatoxylin in Figs. 2 and 3. They may lie anywhere in the cytoplasm, but are more often among the mitochondria and close to the idiozome. In the living material they do not appear in the unstained cells, but when cells are stained with neutral red, they take the stain faintly some time after treatment. There are then four sorts of inclusions in the cytoplasm of the cells in the early stages of the growth period: (1) the mitosome, (2) the idiozome, (3) the fragments called "neutral red granules," (4) the masses of granular mitochondria.

From the stage of the massive bodies the chromosomes uncoil into the leptotene condition. The threads are fine and at first appear as a tangled mass. The monosome is of course an exception to this rule for it remains as a heavy densely staining mass. Shortly after the leptotenes begin to suggest a polarization, the threads become doubled and pass rapidly into the diplotene condition. Here they remain until the growth period is completed and diakinesis begins. In this process of polarization the spherical idiozome in the cytoplasm apparently plays an important part. It is not always visible in the stained material even when the preparations are carefully extracted with a view to making it clear, but in *every case* in which it can be seen in the late leptotene or diplotene stages it is found that the *chromatin threads are polarized towards it.* Two clear cases of polarized diplotenes are shown in Figs. 5 and 6. Both are approximately the same stage, the first from a fixed and mounted preparation,

and the second from a living cell stained with janus green. The first shows the diplotene threads clearly polarized toward the idiozome, while the second the indefinite heavy threads polarized toward a vacant space in the cytoplasm outlined by the mitochondria. After the diplotene stage the idiozome is never visible.

The failure of previous observers to identify an attraction sphere in at least some of the Acrididæ is rather peculiar, for it seems almost certain that it has been figured before. A body of similar appearance, called by Davis a "mitochondrion," is shown in at least his Figs. 31, 34 and 42. In view of the observed behavior of the mitochondria this is of course incorrect, and it seems improbable that it can be a mitosome at the stages indicated. As has been observed above, however, the idiozome is not always visible, and when taken in conjunction with the fact that a clear polarization of the threads is sometimes hard to find, this may indicate that the idiozome is a structure which is sometimes present and sometimes not.

It remains to trace the behavior of the "neutral red granules" during these earlier stages of the growth period. As stated above and shown in Figs. 2, 3 and 4, these granules are larger than the mitochondria, stain densely with hæmatoxylin, and may lie anywhere in the greatest mass of cytoplasm. When the diplotene stage is reached (cf. Fig. 5) we find instead of several granules a single rather large spherical mass lying in a clear vacuole. It is now exactly similar, though smaller, to the chromatoid body of *Pentatoma seilis* described by Wilson. This body stains very heavily with hæmatoxylin, while in the living cell it is practically invisible. When the living material has been treated with neutral red for about an hour, however, it appears as a highly refractive pink drop in the cytoplasm (Fig. 8). Intermediate stages between these two conditions have not been found in the living material, and even in the fixed preparations one usually finds that the cells from the massive body stage on, contain a single chromatoid body in the cytoplasm. Cells are found now and then in which several granules are apparently fused in one mass, or in which two seem in the process of fusion, as in Fig. 3. It is certain at least that the single chromatoid body appears at the expense of the earlier small granules, and this fact makes it

probable that they have become fused into one mass. Occasionally, however, cells appear in which a granule is present in addition to the larger chromatoid body, and I have observed one case in which two equal granules—or chromatoid bodies—were present in a cell at the time of diakinesis. The mass of the two would approximately equal that of the single body usually found at this time. It is possible therefore that the chromatoid body represents simply the enlargement of one of the smaller granules, as suggested by Wilson, who observed a few granules in the cytoplasm in the presynaptic stages of *Pentatoma*. In any case the single chromatoid body in *Rhomaleum* is undoubtedly made up of the material of the granules seen in the cytoplasm of the spermatogonia and early growth period.

Throughout the growth period the chromatoid body increases in size. When the early diakinesis is reached the cell has reached its maximum size, and at this point the chromatoid body is also largest. Fig. 7 shows a cell in which it was even larger than usual, while Fig. 8 represents a living cell at about the same stage, stained with both janus green and neutral red. The mitochondria at this stage are scattered about the cytoplasm to some extent, though they still show a tendency to be aggregated in a mass at one side of the nucleus. From this point onward the chromatoid body behaves as an inert mass, which does not increase in size, and is not affected in the slightest degree by either maturation division. At each division the mitochondria are arranged in the manner described by Lewis and Robertson, except that here again I noticed but little tendency to the thread-like form so evident in *Chorthippus*. The granules are arranged in rows about the spindle but their separate granular condition is always evident. The chromatoid body remains in whichever half of the cell the division plane happens to place it. It is sometimes within the mass of spindle fibers as in Fig. 9, which represents a first spermatocyte telpohase, but more often toward the periphery of the cell. Fig. 10 shows a very late telophase of the second maturation division. The nuclei have already assumed the flocculent appearance characteristic of the early spermatids. The cells are completely divided except for the spindle which still bridges them. The mitochondrial masses

have already become aggregated into the definite spheroidal nebenkerns, and the cytoplasm is clear and free from granules. The chromatoid body, here unusually small, is seen in one of the cells close to the wall of the nebenkern.

It is unnecessary to give details of the metamorphosis of the spermatids. In living material stained with janus green Lewis and Robertson have shown that the nebenkern appears as a mottled spherical mass, which finally divides into hemispheres, between which the axial filament passes. These granular hemispheres elongate as the tail draws out, eventually forming two dotted lines, one on either side of the axial filament. Here again the mitochondria appear as separate granules, with little tendency to fusion even in the mature sperm tail. In a few preparations stained by the Benda method I have noticed the "acrosome sphere," described by Meves and by Montgomery at the opposite side of a spermatid from the nebenkern (Fig. 11). It stains purple, which is the typical mitochondrial reaction. I have been unable to trace its origin or subsequent history, but by analogy with other forms it probably forms the perforatorium of the mature sperm. Since the chromatoid body never divides it should be found in one fourth of the spermatids. In order to test this expectation ten cysts of spermatids in various stages were selected at random from several preparations, and the total number of cells and chromatoid bodies recorded. The results follow:

No. of Cyst.	No. of Spermatid Nuclei.	No. of Chromatoid Nuclei.
1	59	12
2	78	21
3	56	15
4	54	11
5	64	13
6	40	9
7	50	11
8	101	22
9	61	15
10	56	14
Total	619	143

The 143 chromatoid bodies observed are fairly close to the expected number 155.

With the elongation of the spermatid the chromatoid body

wanders further and further from the nucleus, and usually lies close to the axial filament (Fig. 12). It migrates eventually to a considerable distance down the tail, where it may be seen, still in its vacuole, forming a swelling at one side of the axial filament in the nearly mature sperms (Fig. 13). When the metamorphosis is complete no bodies are seen in the tails themselves, but scattered among them are numerous deeply staining bodies of the same size in various stages of degeneration. This condition is plainly visible in the living material where the loose granules stain quickly and brilliantly with neutral red. The process is therefore identical with that in *Pentatoma*, except that it is unusual to find any great amount of protoplasm cast off from the sperm tails with the chromatoid bodies.

Staining Reactions of the Chromatoid Body.

As has been noted above the chromatoid body in *Rhomaleum* gives the specific reaction when the living cells are treated with neutral red which has been described for certain granules of similar behavior in *Chorthippus*. Lewis and Robertson have also observed that somatic and apical cells often appear to be crowded with material which gives a similar though more distinct relation. I am able to confirm their observation with regard to the apical cell in *Rhomaleum*. It appears, therefore, that a specific substance which is an ordinary inclusion of the cytoplasm of certain somatic cells and of the apical cell is present in the cytoplasm of the spermatogonial and early growth period cells of some grasshoppers in small quantities, that it increases in amount during the growth period, and may become aggregated into one mass. Like almost any foreign substance in the cytoplasm this mass appears to lie in a vacuole in fixed material. It is finally eliminated from the nearly mature spermatozoa. That a similar condition occurs in the male germ-cells of many other animals is made probable by the fact that similar bodies have been described in an increasing number of forms.

As to what this substance is, one can give no certain answer. In the living cells it remains almost invisible when unstained, and it appears faintly pink after a rather long treatment with neutral red. In the fixed material the body stains densely with

hæmatoxylin, safranin and other chromatin stains. When the Flemming triple combination is used the chromatoid body is red throughout, even though the resting nuclei are purple. With the Auerbach stain the body is clearly differentiated from the chromatin, for it is bright red while the nuclei are green. With the Benda alizarin-crystal violet method, even when the material is fixed as directed with the modified Flemming's fluid, the mitochondria seldom appear, and the nuclei and chromatoid body appear bright purple. I have tried this method repeatedly with the germ cells of *Rhomaleum* but have never been able to get the brilliant result shown by Giglio-Tos and Granata (1908) in their paper on *Pamphagus marmoratus*. With the Altmann acid fuchsin method the chromatoid body is clear red as are the mitochondria. The material is therefore unlike either mitochondria or chromatin in chemical constitution, a fact clearly shown by its behavior.

I am glad of an opportunity to express my indebtedness to Professor E. B. Wilson for generous advice in the preparation of this paper as well as for numerous suggestions with regard to technical methods. I am also indebted to Miss Mabel Hedge for the original drawings of Figs. 3 and 4.

Summary.

The mitochondria in *Rhomaleum* are shown to be present in the spermatogonia. Their behavior agrees closely with that described by Lewis and Robertson for *Chorthippus*, except that they remain granular throughout.

There are present in the spermatogonial cells of *Rhomaleum* in addition to the mitochondria certain fine granules which stain in contrast to the mitochondria with neutral red. These granules are carried over into the early spermatocytes where they probably become aggregated into one mass. This mass grows for a short period, passes inert through the two maturation divisions, and into one fourth of the spermatids. From the tails of the developing sperm it is cast off into the end of the follicle, where it degenerates. In addition it has been noted that an idiozome or attraction sphere is present in the early spermatocytes of *Rhomaleum*, and an acrosome sphere in the spermatids.

Columbia University,
October 5, 1916.

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Explanation of Plate I.

All figures except Nos. 1, 6 and 8 from camera drawings made with Leitz 1.5 mm. oil immersion objective and 8 compensating ocular. Nos. 1, 6 and 8 from living material with 2 mm. objective.

Fig. 1. Resting stage of secondary spermatogonia—living cell stained with janus green showing granular mitochondria.

Fig. 2. First spermatocyte showing chromatin network with cloudy mass of mitochondria and prominent "neutral red granules" in the cytoplasm.

Fig. 3. First spermatocyte beginning to show the "massive bodies"; mitosome, idiozone and "neutral red granules" visible in cytoplasm.

Fig. 4. Similar to the preceding, the idiozome shrunken so as to appear in a vacuole because of imperfect fixation.

Fig. 5. First spermatocyte bouquet stage—diplotene threads polarized toward idiozome, chromatoid body visible.

Fig. 6. Similar to preceding—living cell stained with janus green—mitochondria in two masses surrounding a spherical space in which the idiozome (invisible) probably lies, since the diplotene threads are polarized toward it.

Fig. 7. Diakinesis—drawn from a preparation stained with alizarin and crystal violet—showing large chromatoid body in a vacuole.

Fig. 8. Similar stage in living cell stained with both janus green and neutral red,—mitochondria and chromatoid body visible.

Fig. 9. Telophase of first spermatocyte division—the chromatoid body passing to one pole.

Fig. 10. Late telophase of second spermatocyte division—spermatid nuclei already formed—mitochondria aggregated into the spherical nebenkerns-chromatoid body in one daughter-cell.

Fig. 11. Spermatid showing nebenkern and acrosome sphere.

Fig. 12. Spermatid, tail beginning to elongate, nebenkern divided, chromatoid body passing down close to the axial filament.

Fig. 13. Group of sperm tails, showing chromatoid bodies just before they are cast off.

FIG. 1.

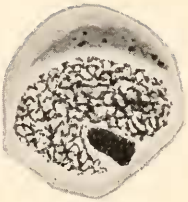

FIG. 2.

FIG. 3.

FIG. 4.

FIG. 5.

FIG. 6.

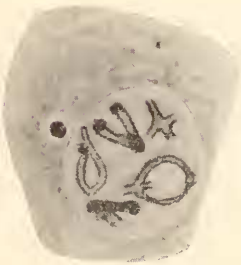

FIG. 7

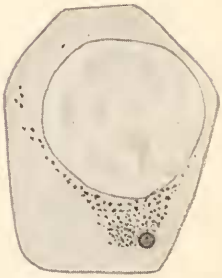

FIG. 8.

FIG. 9.

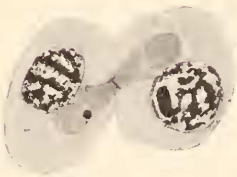

FIG. 10.

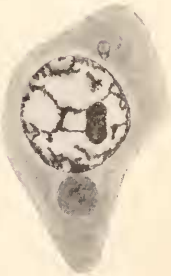

FIG. 11.

FIG. 12.

FIG. 13.

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ON THE BREEDING HABITS OF DESMOGNATHUS FUSCA.

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That the eggs of *Desmognathus fusca* are deposited under terrestrial conditions, and are brooded by the mother during their development, are facts already well known. In my account of the "Life History of *Desmognathus fusca*" (I. W. Wilder, '13), I made the following observation and suggestion: "The eggs are always found guarded by a female, undoubtedly the mother. She usually so places herself among them as to bring practically all of the eggs in contact with her body, which often extends through the mass of eggs and is frequently bent sharply upon itself as if the better to surround and protect them (Fig. 1).

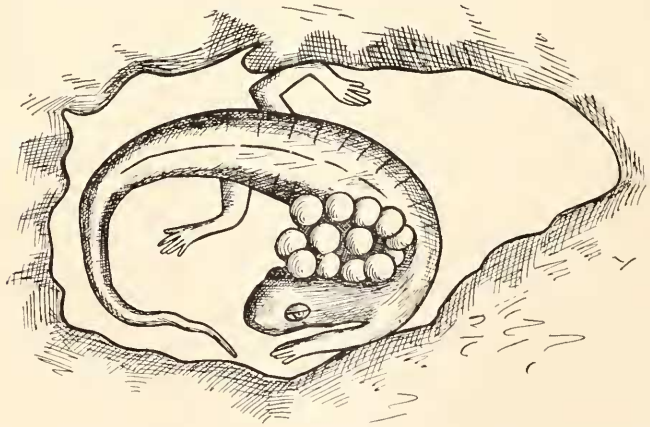

Fig. 1. Female *Desmognathus fusca* with eggs, showing the characteristic brooding position, with the body coiled about the eggs. Drawn from life by H. H. Wilder. From *American Naturalist*, Vol. XXXIII.

When under observation, as in a terrarium, the mother frequently leaves the eggs when disturbed, always retreating through the same exit from the nest. After having been separated from the

eggs, however, as may occur in making a transfer from out of doors to the laboratory, the mother goes back to them again, even though the nest and all of its surroundings may have been reconstructed. I have never had the opportunity to further test the sense of ownership of eggs in a mother by exchanging the eggs of two individuals, but the experiment would certainly be an interesting one."

In connection with later experiments upon the mating habits of *Desmognathus fusca*, certain facts have come under my observation which give more definite information concerning this brooding instinct of the mother and her behavior during the brooding period. These facts furnish, in addition, more exact data concerning the length of the incubation period of the species, which, at the time of the publication of my account, was not definitely known.

On July 2, 1915, late in the afternoon, a female *Desmognathus fusca* which had been under observation in the laboratory since May 20, 1915, confined in a terrarium with another gravid female and a male, was found to have deposited a batch of eggs. These had been laid within the previous twenty-four hours, and were in the early segmentation stages when found. The companion male and female were immediately removed from the terrarium and in order to make sure that the eggs were actually those of the female with whom they had been found associated, the companion female was examined and found to be still gravid.

The terrarium in which the eggs were laid was a rectangular glass one, measuring 13 inches long by 8 inches wide by 13 inches deep, and had a two-inch layer of wet sand in the bottom, sloping down at one corner to allow the water to stand in a shallow pool. Upon the sand had been placed wet sphagnum moss, and a stone about 4 inches in diameter was lying upon the sphagnum near the end of the terrarium which was opposite to the pool. It was in a cavity in the sphagnum underneath the stone that the eggs, 22 in number in two clusters, were found with the mother coiled about them in the usual brooding position of the species.

During the subsequent weeks the stone was frequently lifted to examine the nest, in the evening as well as during the

daytime, and the mother was always found in this characteristic coiled attitude, although the relative position of the eggs and the mother was slightly changed from day to day. She seemed little disturbed by these brief examinations unless the eggs were actually handled, when she would leave the nest and go away a little distance, always, however, to return to her charge later. On August 23, after an incubation period of 52–53 days, the eggs began to hatch, the process continuing through August 25.

Meanwhile, on July 7, in the late afternoon, another female in a similarly arranged terrarium, was found to have begun to deposit eggs. This female had been captured on the same date as the first (May 15), had shown when captured a similar evidence of the presence of ripe eggs conspicuous through the body wall, and had been similarly confined since May 20 with another gravid female and a male. On July 1 the male had been removed, however, and as soon as the eggs were discovered the other female was removed also. At this time a small cluster of three or four eggs had been deposited, and a single isolated one which was removed at once from the nest for examination, and was at this time unsegmented.

On the following forenoon the egg-laying process had been completed, and the usual two clusters of eggs were found, one numbering six and the other twelve. The cluster of six eggs was appropriated for study of the early cleavage, and it was found that at that time, about twelve o'clock, some of them were in the first cleavage stage, while others showed as yet no suggestion externally of the cleavage process. The one which had been removed on the previous afternoon was at this time in the second cleavage stage.

The eggs of this second batch were deposited in a cavity hollowed out in the moist sphagnum under the stone, as was the case of the first batch of eggs which had been deposited in the other terrarium a week earlier. This second mother, however, took from the beginning an unusual position with reference to the eggs, for instead of lying among them with her body coiled about them, she stood over them with her body in a straight line and slightly elevated so that the eggs were beneath her belly and only slightly in contact with it. Care was always taken in

examining the nest not to disturb her or her surroundings beyond lifting the stone carefully from the nest and replacing it with equal care, in exactly the same position. She was invariably found in the same peculiar attitude with relation to the eggs, and always with the head oriented in the same direction.

On August 24, after the eggs of the first batch had begun to hatch, both of the mothers were removed from their respective eggs. The mother of the second batch was killed and preserved. The mother of the first batch, now hatching, was transferred to the terrarium containing the second batch, now motherless, but was placed in the part of the terrarium farthest from the stone under which the eggs were located. Great care was taken to leave the sphagnum surrounding the eggs, and the stone covering them in the same relative position which they had had throughout the incubation period.

On the following day when the terrarium was examined the female was not in sight, but upon lifting the stone, she was found under it brooding the eggs and standing over them in exactly the same unusual position which their own mother had habitually assumed. She remained with them and was always found in the same position, with her body oriented in the same direction, until, on September 2, a week later, all of the eggs had hatched. Upon this date the young were found clinging to the body of their foster mother as she stood with her customary orientation, a number of them surrounding the middle of her body like a rosette, with their long axes parallel to hers but with their heads pointing in the opposite direction. When she was disturbed, a few were somewhat scattered while most of them still clung to her body. After a few days, the adult was found in the little pool at the opposite end of the terrarium, and the young, still in the terrestrial larval stage, were distributed through the moist sphagnum between the nest and the pool.

Of course there is no means for determining whether the actual finding of the eggs by the foster mother was a reaction to the proximity of the eggs themselves, or was a purely accidental occurrence, since *Desmognathus* frequently seeks out positions under stones and other objects lying upon the surface. Obviously, however, in her subsequent week of continual brooding

of the eggs, and in her assumption of the very unusual attitude during this period, we have an interesting example of a perfectly automatic response to external conditions. Otherwise her attitude would hardly have shown, as it did, so exact a correspondence to the aberrant one previously assumed by their own mother, but would rather have been the characteristic one which the foster mother had taken in brooding her own eggs. The chief of the conditions controlling this automatic response would seem to have been the presence of the eggs themselves, since, after the foster mother had once reached the pool, she apparently did not return to the stone again but was always found in or near the pool. The peculiar position and orientation of the bodies of the two females while successively brooding this particular batch of eggs is most satisfactorily explained, however, as a response to some unusual conditions in the surroundings of the nest, such as the possible entrance of a little light into the nest from one direction. This explanation receives some corroboration in the fact that a little crevice was noted leading from the surface into the side of the nest toward which the head of the adult was directed. Furthermore, the newly hatched larvæ, which would be expected to be negatively phototropic if they are to succeed in reaching the neighboring water by working their way down through the moist earth and debris, oriented themselves in the direction opposite to that of the mother.

It is moreover possible that we have a further automatic response exhibited by the mother in seeking the water after the young had hatched. This movement toward the water would be of use to the offspring, for they tend to cling to the mother and would thus be eventually guided by her to the water. Under natural outdoor conditions where the nest would be farther away from the water than was possible within the limited dimensions of the terrarium, the time occupied in making this transfer might roughly coincide with the duration of the terrestrial larval stage.

That the terrestrial larval stage is really a definite one is shown by the behavior of the newly hatched larvæ when placed in the water. They are so well developed muscularly that they can not only swim, but can maintain a horizontal position in the

water when not swimming, instead of lying on one side as do the newly hatched larvæ of most amphibians. Nevertheless they will not remain in the water, but persistently crawl out and lie, often in a mass together, in the moist debris along the edges. It is not until all external evidence of the yolk mass has disappeared that they will remain in the water.

The period of incubation in both of these broods was approximately eight weeks (53–55 days in the first case, and 56–57 days in the second), a considerably longer time than that previously estimated by me, which was five weeks. The terraria were kept in a cool basement room, where the temperature did not vary much from 21° C. (70° F.), which was probably somewhat above the average temperature to which the eggs would have been subjected under natural conditions along the banks of brooks in shaded ravines. The former estimate of five weeks was based upon observations of a batch of eggs which were deposited and developed under still warmer laboratory conditions. As the measurements and descriptions of an embryo from this batch after 30 days' development (H. H. Wilder, '99) show a considerably larger size and a more advanced stage of development than the 34 day embryos of the batch of eggs here reported, one is justified in the conclusion that at the higher temperature development took place more rapidly. On the other hand, it is conceivable that in nature the period of incubation might easily be prolonged to more than eight weeks by the lower temperature to which the eggs would certainly be subjected in the neighborhood of cold, spring-fed, mountain brooks. Thus the batch of eggs previously described by me as having been found in nature hatching on September 24 (I. W. Wilder, '13), after an unusually cool summer, may even have been deposited as early as the middle of July, the month reported by Reed and Wright ('09) as the month of maximum egg-laying for the species. This longer estimate of the period of incubation under natural conditions would account for the usual absence of the larvæ of this species from the brooks during the summer months, a fact which is reported by my colleague, Mr. E. R. Dunn, in an article now in press.

The female which acted as the foster mother in the case here

reported was continued under observation in the laboratory for nearly a year. She was fed abundantly upon *Drosophila*, which, as a "by-product" of genetics experimentation, has proved a valuable laboratory food for adult salamanders. In spite of her well-nourished condition, however, she gave no evidence of the ripening of a new lot of eggs during the following spring and summer, while another female of about the same size (87 mm. in total length), which had not been gravid the previous year, developed under the same care and feeding, large eggs which could be conspicuously seen through the body wall early in the spring. These observations are too limited in number to base any definite conclusions upon them, but they at least suggest that the females of this species do not necessarily produce eggs every year. This hypothesis would also explain the fact that I have found that occasionally females collected in early spring contain no large eggs. The number of offspring in this species is in any case very limited, as shown by the small number of eggs in a batch. These average about 20 in the cases which have come under my observation in western Massachusetts, while the largest number of ripe eggs which I have counted in the ovaries of a single individual is only 28, and the number in a batch may run as low as 14. If in addition to this characteristically small number of eggs produced at a time, the females sometimes fail to produce eggs every year, there would be a still further limitation in the number of offspring. Such a reduction has been made possible only by the high percentage of success in the development of the few eggs which are produced. One of the conditions contributing largely to this success is the large amount of yolk present in the egg, which makes possible the attainment of a considerable size and maturity at the time of hatching in consequence of which the larvæ are better able to take care of themselves. A second condition insuring the success of the offspring is seen in the internal fertilization which insures the impregnation and development of every egg which is deposited. In fact, my experiments have shown that gravid females which are isolated from the males early in the spring and thus fail to become fertilized, do not deposit their eggs at all, and that by the middle of August the eggs are already undergoing rapid resorption.

This fact shows an extreme illustration of the conservation of material in this species, and is quite in line with the conservation shown in the reduction of the number of eggs. It shows a decided advance in comparison with the habits of certain other amphibians such as *Cryptobranchus allegheniensis*, for example, which is prodigal in its egg production, but often uses its own eggs as food (Smith, '07). Finally, it is certain that a most potent contributory factor to the high percentage of success in the development of the offspring of *Desmognathus fusca* is found in the extraordinary constancy and devotion of the mother to her offspring during the incubation period, a devotion no less effective because it is an automatic response.

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THE SYMMETRY OF GRAFTED EGGS IN RELATION TO GIANT LARVÆ FORMATION IN ARBACIA PUNCTULATA.

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INTRODUCTION.

Two or more eggs have been experimentally grafted together by several investigators (Driesch, Goldfarb and de Hahn, 2, 3, 4, 5, 6, 7 and 8), who have also described some of the types of larvæ resulting from such grafts. One of these types, the Riesen-larva, first produced and described by Driesch, has aroused considerable interest particularly with respect to its origin. This larva is distinguished from other fused larvæ, in that it is normal in structure, single, and identical with control larvæ, except for size. According to Boveri (1) and de Hahn (5) such perfect fusion into a single larva can occur only when the axes of the fusing eggs are so placed that they bear the same relation to each other as the blastomeres of the two cell stage of an egg; *i. e.*, the axes and the planes of the two grafted eggs must be parallel and symmetrical.

In studying separate clusters of grafted eggs of the sea-urchin *Arbacia punctulata*,[1] and in *following the development of each cluster through its pluteus larva*, numerous facts were disclosed that did not accord with Boveri's hypothesis of the genesis of the Riesenlarva. A concise statement of these facts is given below,

[1] For technique of grafting, see Goldfarb 6 and 7.

and for the sake of brevity only a few stages in the development of each cluster will be shown, all drawn to the same scale except where specifically mentioned to the contrary, and all drawn from the living specimens with the camera lucida.

Single Larvæ, Axes of the Two Grafted Members Unknown.

Under this heading are included those clusters which developed into single perfect larvæ, and in which the axes at the beginning were either unknown, or could not be definitely ascertained.

Fig. 1 is a foreshortened view of a pair of blastulæ, partially fused together. The diameter of each component when compared with the controls clearly shows that these are two blastulæ and not two half blastulæ. The axis of each member could not be definitely ascertained until the blastopore, or better still until the gut, is differentiated. Fourteen hours after the stage shown in Fig. 1 the pair was transformed into a "single" gastrula, with a "single" invagination shown in Fig. 2. This gastrula developed into a "single" typical larva (Fig. 3), which was essentially unchanged during the next four days. This larva contained one skeleton and one gut, and if its history had been unknown might readily be mistaken for a true single larva derived from a single egg.

Fig. 4 is another double blastula which developed into a "single" large gastrula, and then into a young pluteus containing a single pair of skeletal spicules, and a single gut (Fig. 5). Three days after fertilization, it developed into what appears to be a single perfect larva, shown in end view (Fig. 6). During the next four days there was no essential change except the elongation of the anal arms seen in Fig. 7 from a different view.

Four stages are shown in the development of the next specimen. Fig. 8 shows the giant blastula somewhat foreshortened. Fig. 9 drawn seven hours later, shows the giant gastrula with its single gut a little to one side; fourteen hours later the gut is more sharply to one side (Fig. 10). This gastrula developed into a "single" short and somewhat atypic pluteus, containing a single gut, still a little to one side, and a single asymmetrical skeleton with one side distinctly enlarged (Fig. 11).

In the preceding as well as in many other examples, the grafted pair of eggs developed first into a double blastula and later into a single gastrula. All subsequent development was single. In all of these instances it was impossible to determine the axis of the second member, for only one blastopore and one gut was formed. It is possible that the eggs, in these instances, were by chance grafted to each other in the same relative positions as the blastomeres of an egg, and that as a result of this position the grafted pair developed into a single organism as required by Boveri and de Hahn. That this possibility is very remote will be shown in the following sections. Let me first draw attention to such instances in which the axes of both members are known.

Single Larvæ, Axes of the Two Grafted Members Known.

In this section the axis of each member was definitely established by the definite formation of a gut in each member.

Fig. 12 is a foreshortened view of a nearly equal pair of fused gastrulæ whose embryonic guts and therefore whose axes are about 135 degrees apart. This double gastrula developed into a "single" larva (Fig. 13) which grew into a larva decidedly larger than the controls (Fig. 14). This larva contains certain accessory parts which are not uncommon in fused larvæ, at least in certain stages of their development. There is, for example, a small accessory oral rod at X, which structure is sometimes found in control larvæ. There is also an accessory fold of the gut, making four in place of three characteristic divisions of the gut. This condition is very unusual if not entirely absent in true single larvæ. This example is but one of a series which differ only in minor details and which show that "*single*" *giant larvæ may be formed even when the axes of the two grafted members* are not parallel and not symmetrical. How the two guts are moulded into one, and how the relative position of the axes is changed will be considered later.

Whether the relative position of the axes is or is not permanent, one would expect from Boveri's and de Hahn's hypothesis, that two members, which were clearly not symmetrical, in the specimen shown in Figs. 12 to 14, would not develop into a single larva, which is contrary to our observations.

Another double gastrula is shown in Fig. 15, in which the two guts are unequal and in which the *axes are bent at an angle approximately 45 degrees. This double gastrula developed 24 hours later into a "single" larva*, and normal except for a slight swelling of the body wall in the aboral region on one side. Fifty-four hours later it developed into a very large larva (Fig. 16), whose body, skeleton and digestive tract are "single" and normal except for the hypertrophied bar in the aboral region marked X.

I have records of ten other similar pairs of grafted gastrulæ in which the relative sizes of the guts and the axial angle varied, yet in spite of this asymmetry, all of them developed into "single" larvæ like those just described. Two other pairs are described in the next section (see Figs. 26 and 34).

In these instances at least, the particular angle formed by the axes is not correlated with the formation of Riesenlarva, and in all of them *in spite of marked axial asymmetry, the two members fused into perfect or nearly perfect "single" larvæ, and Riesenlarvæ.*

Single Larvæ by Absorption of One Member.

In all the clusters under consideration *there was no separation of the two members.* Separation frequently occurred especially in agglutinated pairs, but such specimens were of no significance in these studies and are omitted from consideration. All of the "single" larvæ described in this paper had their genesis in a pair of more or less completely and permanently fused blastulæ or gastrulæ.

Many grafting experiments have shown that a resorption of parts often takes place, and it was conceivable that in these experiments resorption or disintegration of one of the members might also have taken place. Before any conclusion can be drawn it must be definitely shown whether resorption or disintegration occurred and the nature and degree of resorption. The following examples will throw some light on this phase of the problem.

Fig. 17 is clearly a fused pair of blastulæ approximately equal in size. During the next twenty-four hours the two developed very unequally, one into a gastrula and then into a young pluteus, while the other ceased developing and then decreased in size

(Fig. 18). During the next two days the non-developing member continually decreased in size, and finally disappeared altogether; its mate progressively differentiated and grew into the "single" larva (Fig. 19) which is larger than control larvæ. This example is typical of a fairly large group in which one member is slowly but completely absorbed and in some instances disintegrated.

A stage between that shown in Figs. 17 and 18 is represented in the next example. This grafted pair consists of a blastula and a somewhat larger gastrula. A free-hand sketch is shown in Fig. 20. During the next twenty-four hours the blastula diminished in size and gradually disappeared, while the gastrula developed into a normal young and single larva (Fig. 21). During the next five days this larva enlarged considerably, accessory skeletal bars and accessory gut appeared, as seen in foreshortened view in Fig. 22. During the succeeding four days besides the expected reduction in size of the larva, a number of accessory skeletal bars also disappeared from both anal and arm rods, making the larva resemble even more closely single control larvæ.

In the next example a blastula is grafted to a gastrula (Fig. 23). The blastula gradually diminished in size and disappeared without further differentiation, while the gastrula grew and differentiated into a "single" larva. This larva has a single gut bent a little to one side, with a single skeleton plus an irregular accessory rod connected to the basal and aboral rods of the skeleton (Fig. 24). During the next four days, while the larva continued to grow, two accessory parts of the gut appeared making a five part digestive tract in place of a tri-partite gut as in normal animals. The hind and mid guts are in duplicate. The accessory bar is also enlarged and is much fenestrated. In this example as well as in the preceding, one of the grafted members clearly disappeared BUT DURING AND AFTER THE DISAPPEARANCE OF THIS MEMBER ACCESSORY PARTS APPEARED IN THE SKELETON AND IN THE GUT OF THE DOMINANT MEMBER.

Such accessory parts appeared in all or nearly all cases after one member disappeared, and was frequently associated with a general enlargement of the body. Accessory parts are not rare among controls, but they do not occur as frequently nor are they of the character, nor are there so many accessory parts in a single

organism as in these fused larvæ. These and other facts (see Goldfarb 7 and 8) appear to me to indicate that one of the members is not disintegrated, nor merely absorbed. It appears to me that the cells of the smaller or weaker or more slowly differentiating member are translocated, into the dominant member, where the translocated cells are regrouped into additional gut, skeleton or body wall, and in cases of incomplete regulation, into accessory parts.

Absorption and translocation of cells of one member may take place even after both members have fully differentiated their guts, and the axes of both members was definitely fixed. For example, two grafted eggs developed into a double fused gastrula, one somewhat larger than the other and with axes about 90 degrees apart. A free-hand sketch is shown in Fig. 26. During the next twenty-four hours the smaller gastrula was gradually absorbed, while the other continued its normal development. The resulting larva which is shown in Fig. 27 consists of one body, one skeleton, all normal except for the small accessory bars at the aboral swollen end of the body. During the next two days the larva became decidedly larger, the swelling at the aboral end smaller and concomitant with these changes, two accessory parts of the gut appeared (Fig. 28). During the next three days while the body gradually diminished towards the normal, THE ACCESSORY GUTS LIKEWISE DISAPPEARED AS WELL AS THE ACCESSORY SKELETAL PARTS, *transforming the larva into a completely normal one* (Fig. 29), indistinguishable from control larvæ.

In this instance the two fused gastrulæ, although their axes were 90 degrees apart, gave rise to a "single" larva, as did the paired gastrula of the preceding section. *If the intermediate steps in the development had not been observed, one might have concluded either that this larva was derived from a single egg, or from a symmetrical pair of eggs as required by the Boveri-de Hahn hypothesis.* But it is evident that *the process was quite different, that there was first a gradual and definite absorption of one member, a translocation or migration of parts, an increase in the volume and number of parts of the dominant member, and finally a loss of materials and parts.*

These successive changes do not always give rise to normal

larvæ. Atypic or incomplete larvæ may be formed. I have many records of the transformation of pairs of grafted gastrulæ into atypic enlarged or incomplete single larvæ, which will be described elsewhere. They are the resultant, from the present evidence at least, of a disturbance in the translocation and rebuilding of cells that make up the skeleton, rather than a resultant of asymmetrical positions of the fused members.

Change of Axes During Development.

In studying the development of fused gastrulæ, it became evident that in many instances the relative position of the axes was definitely altered, so that the angle formed by the two guts and therefore the symmetry of the two fused members, was profoundly altered during development. For example, in Fig. 30, two nearly equal gastrulæ were fused in such a manner that, though their blastopores are nearly united, their guts diverged about 60 degrees from each other. During the next six hours, besides increasing in size, and besides an unequal growth of the two guts, the relative position of the *axes had shifted from 60 to nearly 80 degrees* (Fig. 31). This double gastrula gave rise, by the process of absorption, to a "single" larva, with a somewhat incomplete skeleton and single gut (Fig. 32).

Fig. 33 is a drawing of two fused gastrulæ twenty-four hours after fertilization. One member has its gut fully formed, the other member has just begun to differentiate it. Their axes are about 90 degrees apart. During the next seven hours two changes took place, firstly, the smaller grew relatively faster and became as large as its neighbor, secondly, THE RELATIVE POSITION OF THE TWO GUTS HAS SHIFTED FROM ABOUT 90 DEGREES TO 130 DEGREES (Fig. 34).

In the next specimen the two equal gastrulæ are fused in such a manner that their axes were about 140 degrees apart. Fig. 35 is a free-hand drawing of this pair. During the next two days the gastrulæ developed very slowly and unequally into a nearly full-grown pluteus, and a gastrula just beginning to differentiate its triradiate spicules. *Their axes had in the meanwhile shifted from about 140 degrees to 180 degrees* (Fig. 36).

The next example consists of a pair of somewhat unequal

gastrulæ fused so that their axes are about 135 degrees apart. Fig. 37 is a free-hand drawing of this pair. These gastrulæ developed into two fused larvæ *whose axes had rotated from 135 to about 170 degrees* (Fig. 38). In this pair of larvæ it is interesting to note that one half of one member has been completely suppressed.

In the following pair of gastrulæ the AXES ROTATED IN THE OPPOSITE DIRECTION, AND BECAME SECONDARILY PARALLEL, having shifted from about 70 degrees to 0 degrees. The gastrulæ were nearly equal in size and their axes diverged about 70 degrees (Fig. 39). During the next twenty-four hours no material change occurred either in size or differentiation of parts, BUT THE GUTS HAD BECOME PARALLEL (Fig. 40), and ALTHOUGH THESE GASTRULÆ WERE NOW PARALLEL THEY DID NOT FUSE INTO A "SINGLE" LARVA as required by Boveri's hypothesis. It might be urged that while the axes were parallel the two gastrulæ were not necessarily blastomerically symmetrical. Inasmuch as I failed to observe the skeletal spicules which would have established the planes of each of the members, I can offer no opinion, in this example.

In another example a completely differentiated gastrula was fused to one just beginning to differentiate its gut (Fig. 41). This pair is like the one shown in Figs. 33 and 34. Two days later the pair developed into two fused larvæ in which the axes had changed from about 100 degrees to 40 degrees (Fig. 42). This pair is also interesting because one individual developed into a perfect larva, while the other developed into a perfect half larva, the two having a common foregut.

Still more interesting is the pair of gastrulæ shown in foreshortened view in Fig. 43. The axes are about 180 degrees apart, nevertheless during the next seven hours THE AXES HAD SHIFTED FROM ABOUT 180 DEGREES TO 0 DEGREES (Fig. 44). Unlike the pair shown in Fig. 40, these *gastrulæ tended to approach a symmetrical position, yet in spite of this, developed into two larvæ*, one of which was quite irregular (Fig. 45). Much to my surprise the axes continued to rotate during late differentiation, but the skeleton and the gut rotated unequally, for the skeleton shifted from 0 to about 80 degrees and the gut only to about 45 degrees.

In the next example also, the axes tended to approach a parallel position, but the blastopores were 180 degrees apart. The two gastrulæ had their axes originally about 60 degrees apart (Fig. 46). The next day with the development of both retarded, their axes had shifted to about 180 degrees apart and their size and degree of differentiation were more unequal (Fig. 47). This pair developed into a "single" somewhat irregular larva.

Fig. 48 is another instance of axial rotation towards the zero point. This figure is a free-hand drawing of two nearly equal gastrulæ whose axes were 180 degrees apart. The next day the axial angle had shifted about 75 degrees. This angle was maintained throughout subsequent development. The resulting double larva is shown in Fig. 49.

It will be evident even from these few examples that the relative position of the axes may be, and had in fact been shifted during early development of the gastrulæ; that the shifting took place towards or away from blastomeric symmetry of the axes, and that the range of movement was surprisingly large, from about 20 to 180 degrees on the one hand and from 180 to 0 degrees on the other.

It is conceivable that with such shifting of the axes, an original asymmetrical pair may subsequently become symmetrical, and we should then expect from Boveri's hypothesis that development would then be single and Riesenlarva would result. But such Riesenlarva may or may not come out of such symmetrical pairs. That Riesenlarva may be formed in this way cannot be denied. But that there is any necessary relation between symmetrical position of the axes and Riesenlarva formation, is extremely doubtful in view of the examples mentioned above.

On the other hand, if symmetry is not established either by rotation of the axes or by the original position of the pair, we should not anticipate the formation of single larvæ. But as I have shown, such results do occur in at least the four cases described in Figs. 30 to 32, 26 to 29, 15 to 16, 12 to 14.

There is of course the possibility that while the axes may be parallel and symmetrical yet the planes through these axes may not have been parallel, and as de Hahn points out correctly, in the absence of symmetry of the planes, the two members are not really blastomerically symmetrical.

If on the other hand there is but a single example of the development of a Riesenlarva from asymmetrical members, it suffices to overthrow Boveri's theory. And it is unnecessary to demonstrate that two grafted members must be symmetrical. But in four examples at least the axes and planes were clearly asymmetrical, yet they developed into Riesenlarva. At least five others were cited in which the axis of only one member was known, but which also developed into single larvæ. And it is highly improbable that in all these instances the axis of the second member should by a rare combination of circumstances have been blastomerically symmetrical with the known member.

From these results it must be evident that not all Riesenlarva are formed by the fusion of blastomerically symmetrical eggs. While Riesenlarva may be formed in this manner, my observations lead me to conclude that the more common method is by a resultant of many and complicated processes which may briefly be summarized as follows: (1) One member develops normally and completely, the other is arrested in its development, rarely proceeding beyond the gastrula stage. (2) The arrested member is subsequently absorbed, very gradually, and some or all of the cells translocated. (3) The translocated mesenchyme cells form additional skeletal material either making a giant skeleton possible or forming accessory skeletal bars or rods. A translocation of endoderm cells also, either helps in the making of a giant gut, or in forming accessory parts to the gut. (4) With reduction in size, consequent upon starvation of the plutei in the later stages, some or all of these accessory parts tend to disappear. The result of all these changes is frequently a single typical Riesenlarva or atypic double larvæ. In other words the factors making for complete regulation are either not associated with original symmetry of the grafted pair, or with any subsequent symmetry, or they play a very minor role.

Of far greater importance is: (1) the stage in development when fusion takes place; (2) and inequality in the fusing pairs. The earlier the fusion the more complete is it, and the greater the tendency to perfect fusion and Riesenlarva formation. This was in part observed by both Driesch and de Hahn, and Goldfarb. At least any difference in the two fusing members either in size

or rate of development, or possibly in vigor or metabolism, is almost certain to be followed by the train of events just enumerated.

It might still be urged that Riesenlarva are formed only when the eggs are blastomerically symmetrical at the moment of their agglutination or fusion; that if the fused pair become symmetrical at any later time, as was shown to be possible, such symmetry was of no avail. But since there is no definite means of determining the polarity of the sea urchin eggs at the time they are definitely fused together, no positive data can be given for or against this possibility, with this material.

Several instances were shown in which asymmetry from the earliest observable moment was followed by single larva formation, and symmetrical pairs did or did not result in single larvæ.

And finally a word may be said about the polarity of the grafted members. That there is a shifting of the axes, involving the body wall, the gut and the skeleton, has already been pointed out. That mesenchymal and endodermal cells may be shifted was shown by Driesch and Goldfarb, but I cannot translate these changes as effecting the polarity of either member.

Following the absorption of the connecting ectodermal wall between the body cavities of the two members, the pair tend increasingly to reach a state of form equilibrium. This involves the migration of the mesenchyme and endodermal cells, with the consequent change in position and shape of the several structures. There is a mechanical shifting of some cells but not a change of polarity of the cells or of the embryo.

Summary and Conclusions.

1. The development of each pair of fused blastulæ of the sea-urchin *Arbacia punctulata*, was studied separately, and some of these pairs gave rise to single larvæ (Riesenlarvæ).

2. Such larvæ are not consequent upon blastomeric symmetry of the two fusing members, as urged by Boveri and de Hahn.

(*a*) The axis of each member is first definitely known at the gastrula stage.

(*b*) One or both members may differentiate their guts and thereby establish the axes.

(*c*) Single giant larvæ may develop when only one gut was differentiated and only one axis formed in the double gastrula.

(*d*) Single giant larvæ may develop when both guts are differentiated and both axes are known.

(*e*) When these axes are clearly and definitely NOT PARALLEL AND NOT SYMMETRICAL the pair nevertheless gave rise to single giant larvæ.

(*f*) Vice versa, when the axes were parallel single giant larvæ were not developed, but various types of double fused larvæ.

3. It was ascertained that the axes of the two fusing members are frequently shifted and rotated towards or away from blastomeric symmetry and with a remarkably large range of movement; that this shifting towards symmetry had no effect upon the formation of single larvæ; and finally that this rotation of the axes did not affect the polarity of the fusing members.

4. The history of the changes in fused members showed that Riesenlarva may be formed when two fusing eggs are not blastomerically symmetrical; that a complex series of changes take place independent of such symmetry; that these changes are associated with an inequality in the two grafted members, an inequality in size or rate of differentiation, or vigor; that there is a definite tendency for the smaller or slower or less vigorous member to be suppressed in its development, that part or the whole of this member may be absorbed, that a translocation of cells may take place and may develop accessory parts or form enlarged organs in the dominant member. That with starvation there occurs a partial or complete absorption of accessory parts, and reduction in size of the larva. The result of these complicated series of regulatory changes is sometimes the formation of a single giant larva, or a single normal size larva, which, if its history were not known, could not be distinguished from control larvæ.

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'14 Experimentally Fused Larvæ of Echinoderms with special reference to their skeletons. Part 1. Publication 183, Carnegie Institution, 1914, p. 103.

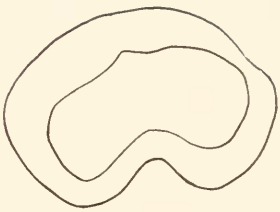
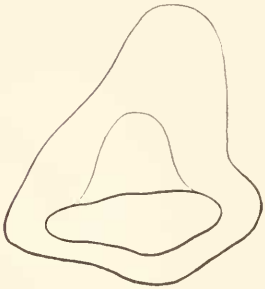


FIG. 1.



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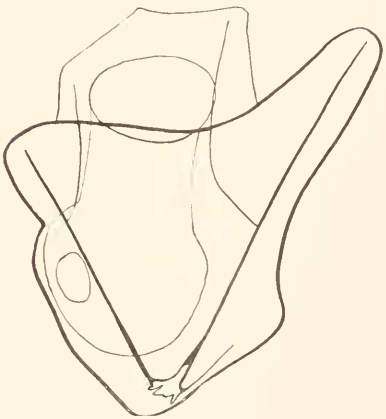

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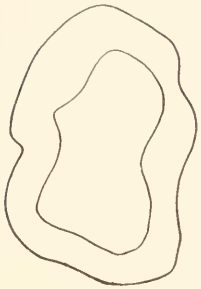

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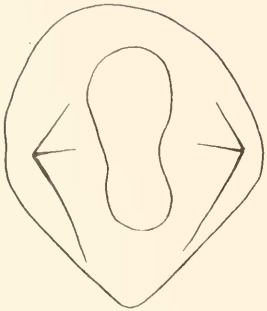

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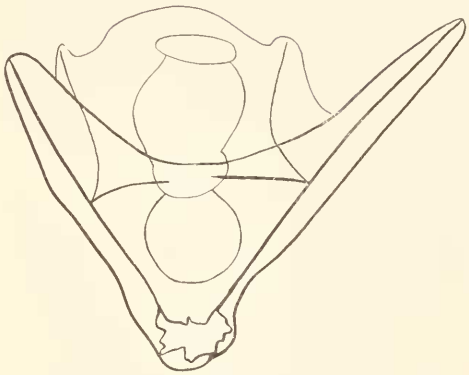

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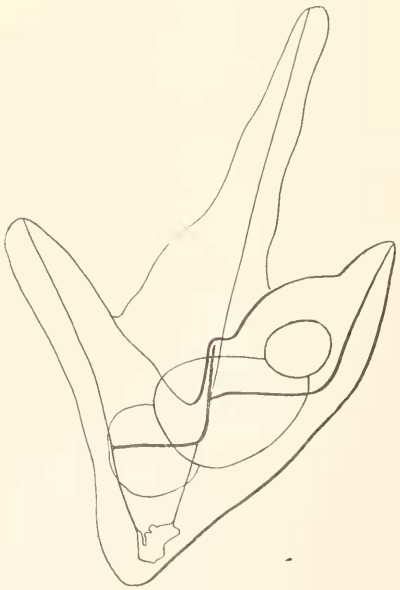

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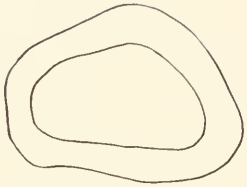

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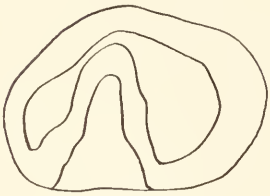
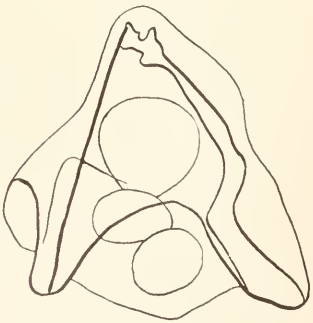


FIG. 9

FIG. 10.



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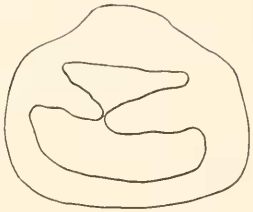

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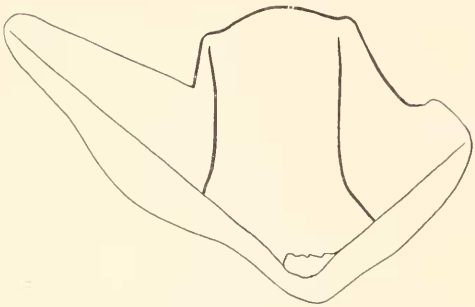

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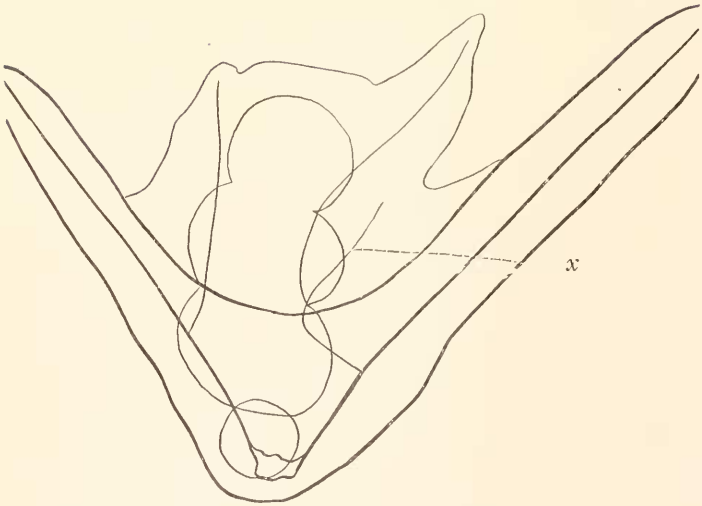

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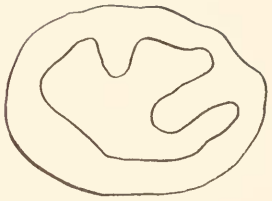

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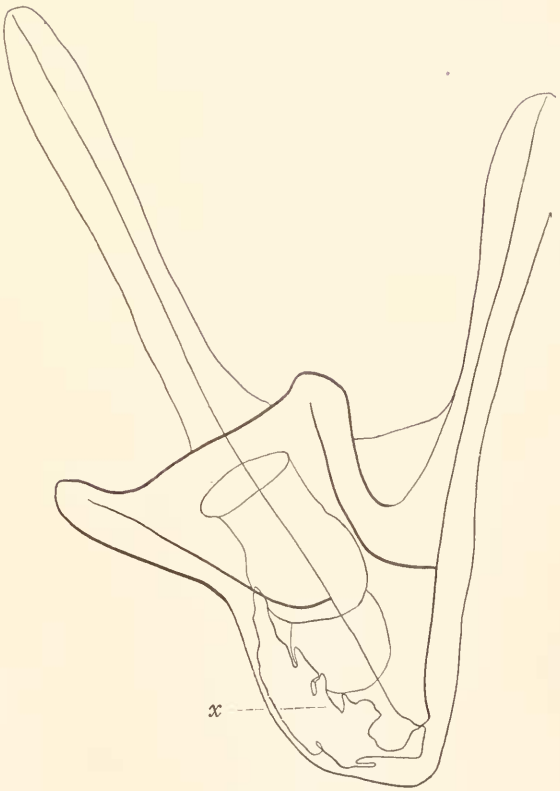

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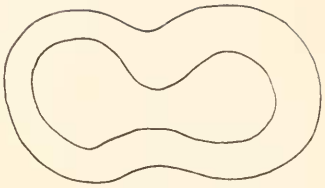

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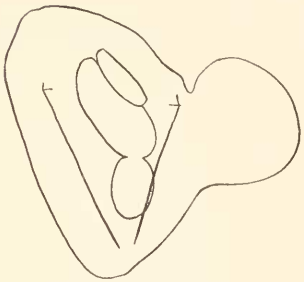

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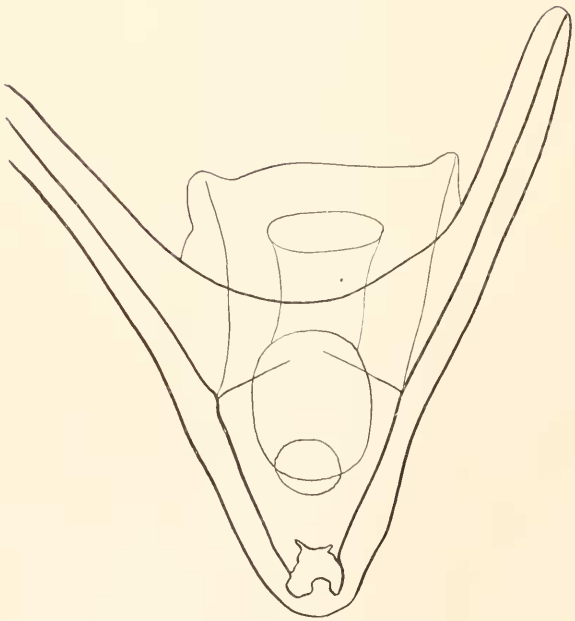

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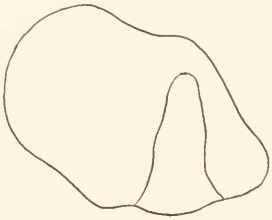

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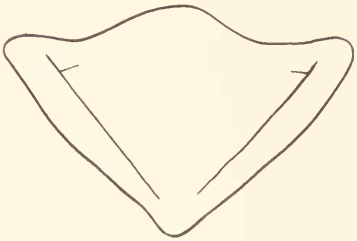

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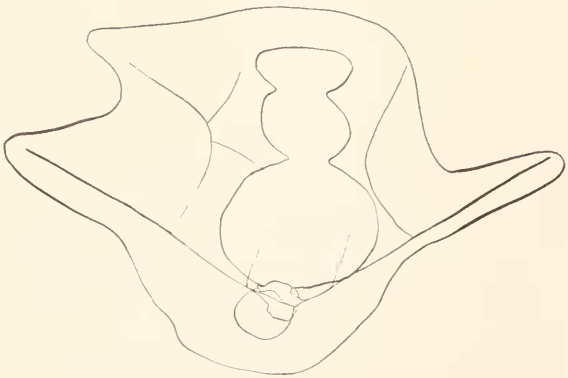

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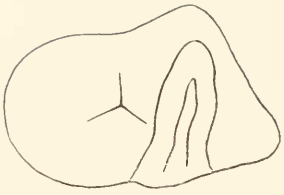

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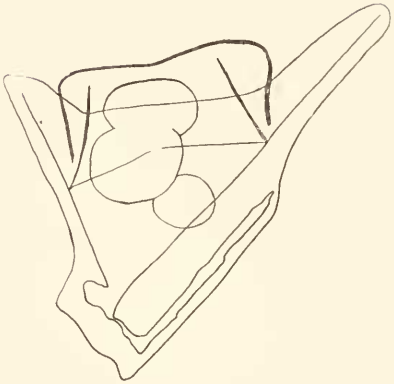

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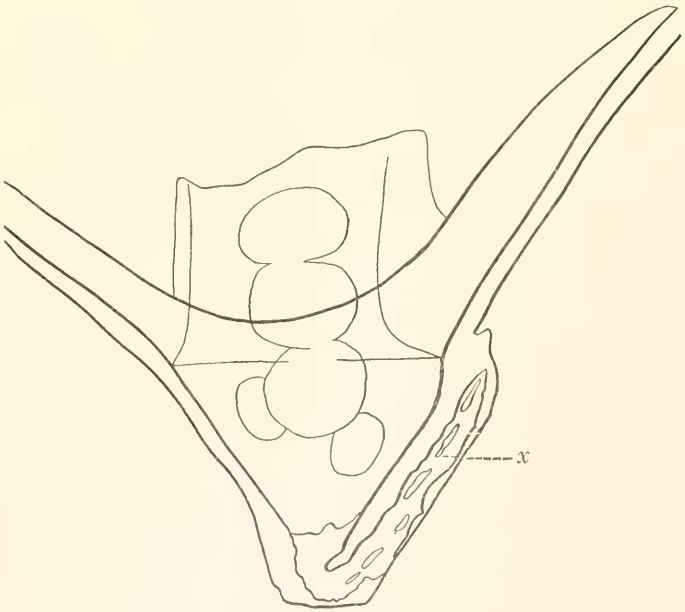
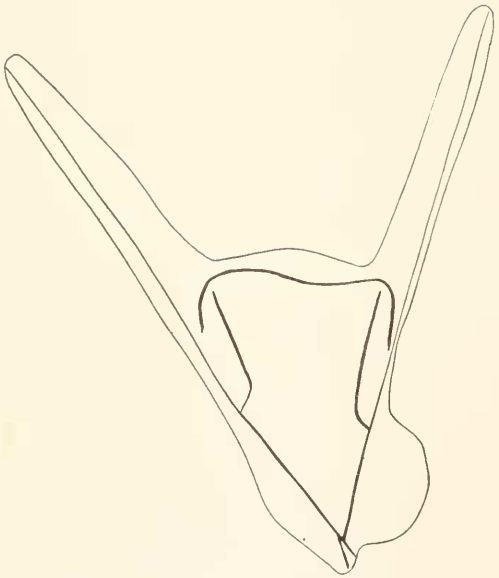


FIG. 25.

FIG. 26.



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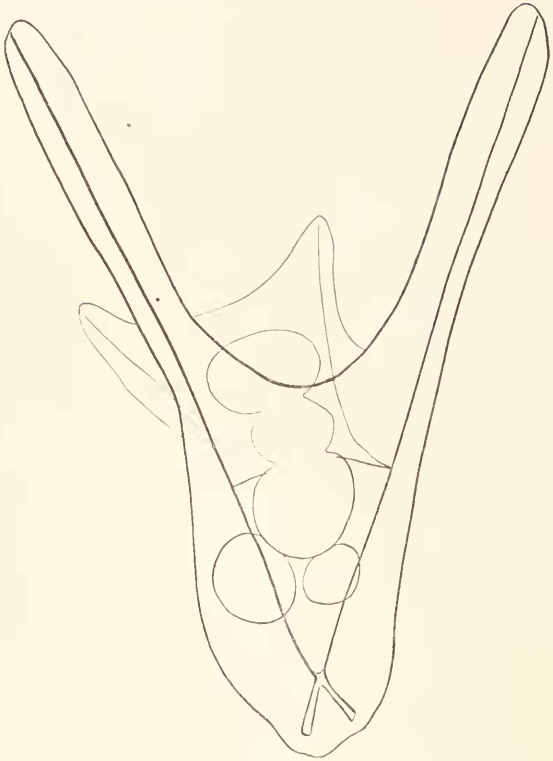

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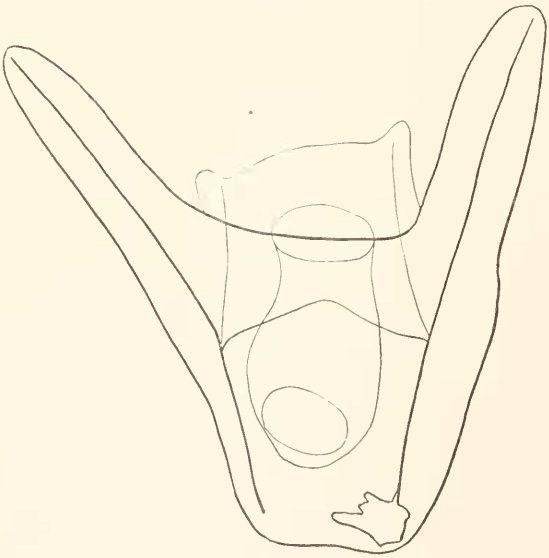

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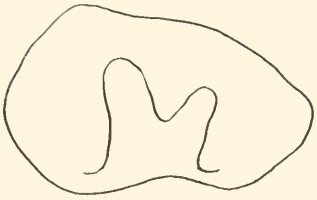

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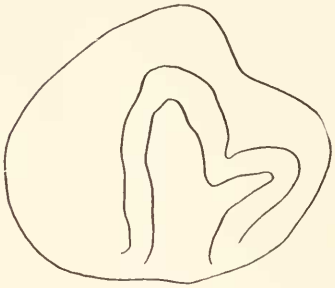

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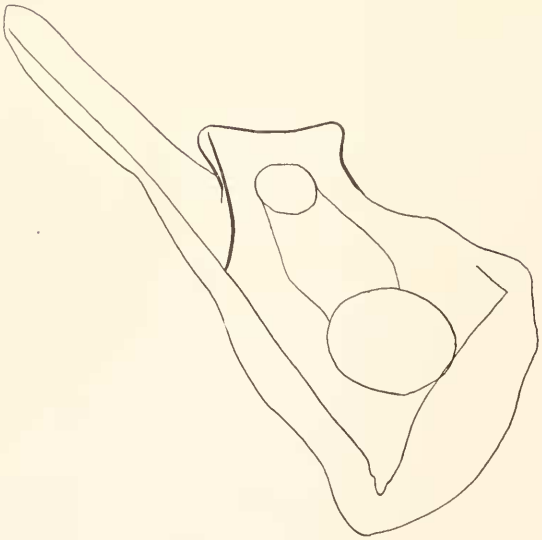

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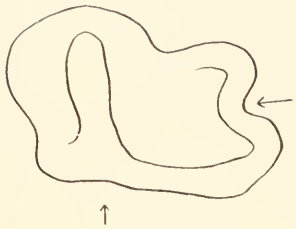

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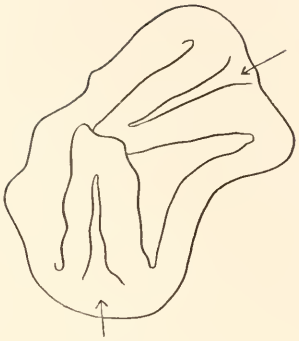

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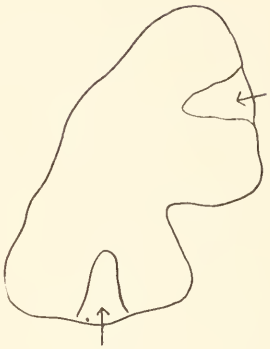

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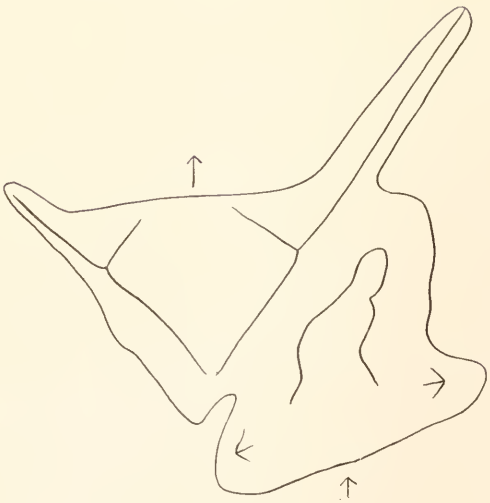

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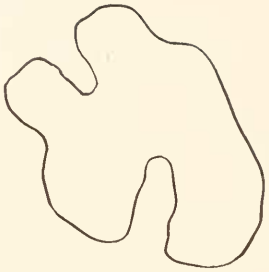

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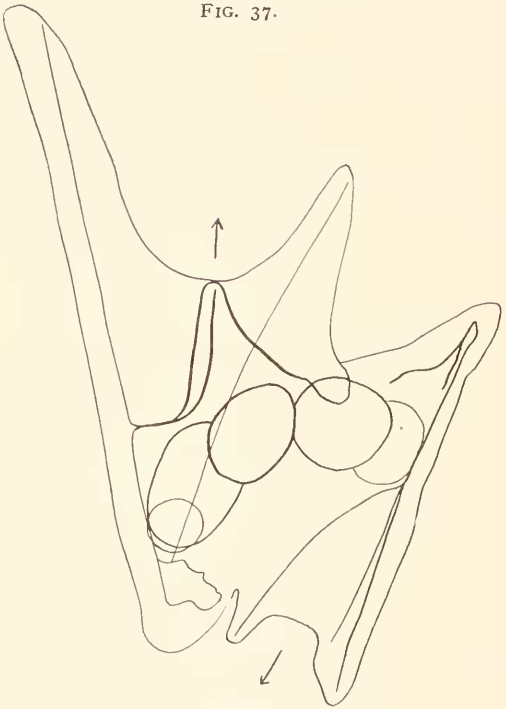

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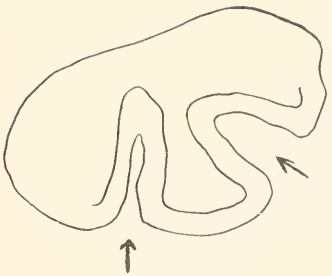

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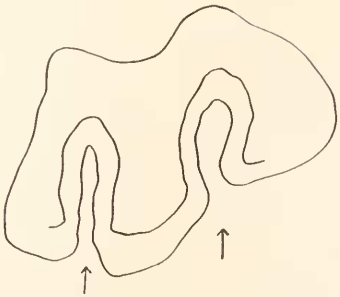

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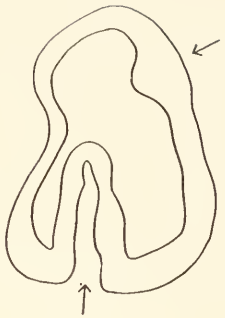

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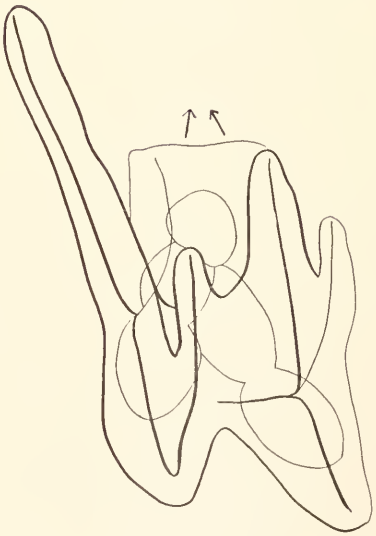

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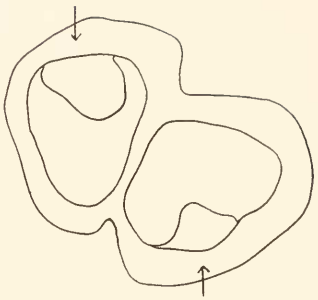

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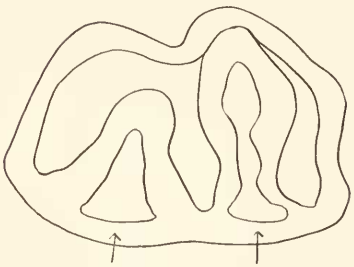

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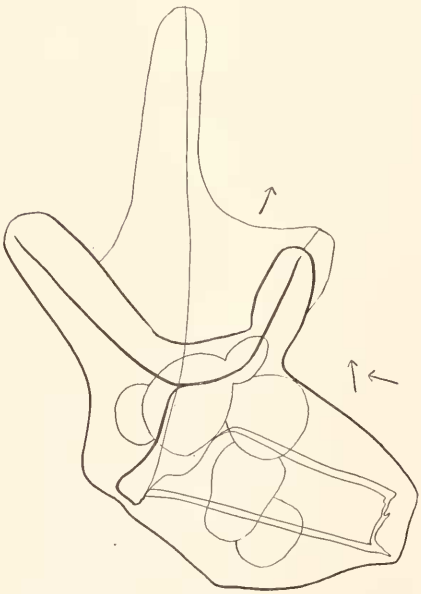

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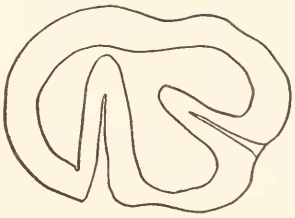

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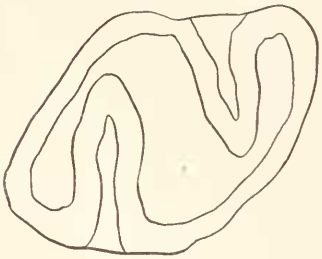

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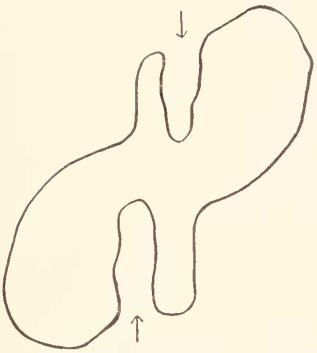

FIG. 48.

FIG. 49.

ON A CASE OF FACULTATIVE PARTHENOGENESIS IN THE GYPSY-MOTH LYMANTRIA DISPAR L. WITH A DISCUSSION OF THE RELATION OF PARTHENOGENESIS TO SEX.

RICHARD GOLDSCHMIDT,

KAISER-WILHELM INSTITUT FÜR BIOLOGIE, BERLIN.

In the discussion about the relation of parthenogenesis and sex, revived after the discovery of the sex-chromosomes, cases of facultative parthenogenesis in normally bisexual animals play a rather doubtful part. As the great majority of such reports belongs to the older and oldest literature, modern writers appear sceptical in regard to their reliability. Therefore any new case recorded under reliable conditions must command the attention of biologists.

The majority of the reports about facultative parthenogenesis relate to the order Lepidoptera. In most cases only the hatching of caterpillars from unfertilized eggs has been observed, which is of no interest for us here. There are comparatively few cases where adults have been raised and their sex stated. One set of observations relates to the silk-moth. This case can be regarded as settled. There can be no doubt to-day that there are races of the silk-moth which exhibit regularly the phenomenon of facultative parthenogenesis and that the normal number of both sexes is produced from parthenogenetic eggs (for example, see Hartmann, '12). Another set of facts relates to the Psychidæ, where parthenogenesis is a normal phenomenon, resulting in female offspring. A third series of reports deals with occasional cases of parthenogenesis in the gypsy-moth, *Lymantria dispar* L. In 1870, H. Weijhenberg, Jr., reported that he had succeeded in breeding 27 adults, 14 ♀ and 13 ♂, from 60 virgin females of this moth. He also obtained a second parthenogenetic brood, but he does not give details (see Dohrn, '71). A second report comes from G. Platner ('88). He claims to have obtained parthenogenetic eggs regularly by keeping the females isolated for a

prolonged period. He states, furthermore, that the maturation-divisions in these eggs are normal. However, a full report of this work never appeared. Since that time many investigators have tried to get parthenogenetic offspring from the gypsy-moth, always in vain. I know that many of those who worked experimentally with the gypsy-moth have tried it again and again, without any success. We and some of our students have used most of our odd material in females for this purpose during the last seven years. The complete failure in every case made us, as well as other observers, believe that the old reports must be based on experimental errors. Nevertheless they seem to be true, for I finally, in 1915, succeeded in getting a parthenogenetic egg-batch.

In this case the conditions of the experiment excluded the possibility of error. I had isolated, for a selection experiment, 4 caterpillars, which were kept in a fruit-jar with a tightly screwed tin cover. After pupation three pupæ died from polyhedra disease. The fourth, a female, hatched. As the experiment was spoiled I left this individual in the closed jar, intending to kill it later. When I opened the jar after a few days the female was busy laying a regular normal egg-sponge, which is normally never done by unimpregnated females. From this parthenogenetic egg-batch, containing certainly over 200 eggs, 22 caterpillars hatched in the spring 1916 and were bred with special care. Three died in early stages and the sex could not be ascertained. Three died before pupation. They were females. Three were killed between the third and fourth moult for cytological study, and proved to be one female and two males. The remaining thirteen hatched and were three females and ten males, the total thus being seven females, twelve males and three unknown. The three females were tried again for parthenogenesis and one of them after ten days finally laid a normal-looking egg-batch. There can, therefore, be no more doubt that facultative parthenogenesis occasionally occurs in the gypsy-moth and results in the production of both sexes. We do not, however, know whether favorable external conditions or some hereditary or not hereditary quality is responsible for the occurrence. The parthenogenetic mother was, in our case, an

F_1-hybrid between a European and a northern Japanese race of gypsy. But neither the parental races nor the other F_1 and F_2 individuals produced parthenogenetic eggs, although ample opportunity was given to many isolated females. This fact neither excludes nor favors the possibility of parthenogenetic strains or mutations.

In order to give an interpretation of these facts it is very important to know the behavior of the chromosomes of these parthenogenetic eggs. A study of the maturation divisions was, of course, impossible. But we were able to ascertain that ovogonia, as well as spermatogonia, of the parthenogenetic caterpillars contained the normal (diploid) number of chromosomes. A visible difference between the chromosome-sets of the two sexes does not, however, exist in the gypsy-moth. The literature on parthenogenesis contains, so far as we are aware, only two statements which relate to our case. One is Platner's already quoted paper, where he states that the reduction-divisions in parthenogenetic dispar-eggs are normal. However, he does not mention the chromosome numbers and we do not know whether the eggs studied by him would have developed. Henking ('92) studied the reduction-divisions of parthenogenetic silk-worm eggs and found a normal reduction-division. But his eggs never developed embryos; therefore his results are of no value for us.

For a real understanding of the relation of parthenogenesis to sex it is very important to know how the diploid number in other parthenogenetic animals is formed. If we compare our case with others where the cytology of parthenogenesis has been worked out, we immediately realize that there are different possibilities. In the first place, parthenogenesis could occur without a reduction-division, as seen in aphids and other forms. Or parthenogenesis could be started after a reduction-division by secondary fusion of the egg-nucleus and the reduction-nucleus, as has been shown for *Artemia* and the starfish. Or, thirdly, an apparently normal formation of the polar bodies could occur, but without reduction of the chromosomes, caused by their failure to conjugate, as has been shown for *Nematus* (Doncaster, 1906) and *Rhodites* (Schleip, 1909). Finally, it is possible that, after normal reduction-divisions, the diploid number is restored before

segmentation by a rudimentary division. No case of this kind has been proved beyond doubt.

The current ideas about the relation of parthenogenesis and sex are primarily concerned with the chromosome number and with an eventual extrusion of a single sex-chromosome. If these conceptions are valid, the different methods of reaching the diploid number of chromosomes would not affect the resulting sex. But, at the same time, these conceptions have failed to explain why parthenogenesis produces only males or only females or both sexes, and that sometimes with, sometimes without, reduction. The ideas about sex-determination which we have developed during the last few years enable us, as we believe, better to understand the different facts about parthenogenesis and to fit them into the general scheme of sex-inheritance.

Since we stated our views in a general way not long ago (Goldschmidt, 1916), we do not need to repeat them here in extenso. We might mention only that we believe to have proved (1) that there are different sex-factors for the sexes, both acting independently in both sexes; (2) that both factors exhibit a definite quantitative action; (3) that the definitive sex depends upon which factor has the higher value, or, expressed in a formula, $F - M > e = ♀$, $M - F > e = ♂$; (4) that one of these factors is carried in the sex-chromosome, the mechanism of their distribution—or, in Mendelian symbolism, of the gamete-formation in heterozygosis—being the means of regulating the values for e in favor of F or M respectively; (5) that the factor not carried in the sex-chromosome, namely F in the case of female heterozygosis, M in male heterozygosis, is inherited maternally, probably in the protoplasm of the egg.

Thus the conclusions which we must draw concerning the relation of parthenogenesis and sex are of course different from those of older writers. Let us first glance at the possible combinations to be derived from our conceptions. For convenience we use the formulæ: $(FF)\,Mm = ♀$; $(FF)\,MM = ♂$, in the case of female heterozygosis, and $(MM)\,Ff = ♂$, $(MM)\,FF = ♀$, in the case of male heterozygosis. And we keep in mind the fact that the factors within the brackets are inherited maternally and are, therefore, contained in every egg, the others being carried

by the x-chromosomes and following their distribution. The possibilities are now as follows:

1. Female heterozygosis. ♀ = (*FF*) *Mm*, ♂ = (*FF*) *MM*.
   - *A*. Parthenogenesis occurs with the reduced number of chromosomes. Offspring must be female as no set *MM* can be produced.
   - *B*. Parthenogenesis occurs with normal number of chromosomes in consequence of no reduction-division taking place. All offspring are female, as the maternal combination is preserved.
   - *C*. Parthenogenesis occurs with the normal number of chromosomes, reached by readjustment after reduction.
     - *a*. Readjustment accomplished by conjugation of egg and polar nucleus. All offspring female, since maternal combination remains.
     - *b*. Readjustment accomplished through rudimentary division before cleavage. The reduction had led to eggs with *M* and eggs with *m*. *M* eggs then become *MM*, *i. e.*, males, *m* eggs become *mm*, *i. e.*, females, if viable at all.

Conclusion.—Parthenogenesis with female heterozygosis can result in the production of (*a*) females exclusively (cases *A*, *B*, *Ca*); (*b*) males exclusively (case *Cb* when *mm* eggs not viable); (*c*) both sexes (case *Cb* if all eggs are viable, or any combination of *Cb* with the other cases).

2. Male heterozygosis. (*MM*) *FF* = ♀; (*MM*) *Ff* = ♂.
   - *A*. Parthenogenesis occurs with the reduced number of chromosomes. Offspring nothing but males. However, the occasional formation of females is possible when a case of non-disjunction occurs, leaving both *FF* inside the egg.
   - *B*. Parthenogenesis occurs with the normal number of chromosomes in consequence of the failure of reduction. The maternal combination being preserved, all offspring are female. In this instance males can be produced if one *x*-chromosome is extruded during the equation division.
   - *C*. Parthenogenesis occurs with the normal number of chromosomes reached by readjustment after reduction.

a. Readjustment through conjugation of egg and polar nucleus. The maternal combination being preserved, only females are produced.

b. Readjustment through rudimentary division before cleavage. Only female offspring result, since every egg contains *FF*. In case of non-disjunction, an exceptional male may appear, provided an *ff* egg is viable.

Conclusion.—Parthenogenesis with male heterozygosis can result in the production of (*a*) females exclusively (cases *B*, *C*), exceptional males explained by *Cb* or the occasional occurrence of *A* or *B*; (*b*) males exclusively (case *A*), exceptional females explained by non-disjunction or the occasional occurrence of *B*, *C*; (*c*) both sexes (case *B* or combination of *A* with *B* or *C*).

We may now, by surveying briefly the facts known about parthenogenesis, show that the above explanation holds good for all of them.

1. *Hymenoptera.*—The classic case of the bee is of special interest because it demonstrates the possibility of sex-differentiation without the use of the usual method of the formation of two kinds of gametes. It is important also because it shows that we are entirely at a loss if we express ourselves in Mendelian symbols without referring to the cytological facts. Parthenogenetic eggs produce males which develop with the reduced number of chromosomes.[1] We are concerned with case 2*A* of the series above described. In spermatogenesis no reduction occurs and only one kind of spermatozoa is formed, being in constitution identical with the ripe egg. Every fertilized egg, therefore, develops into a female. Occasional females derived from parthenogenetic eggs—reported from time to time—(the same for ants)—can be explained by non-disjunction (2*A*) or the occasional occurrence of 2*B*, *C*. Occasional males from fertilized eggs are also possible if a non-disjunction egg (with both *FF* in the polar body) is fertilized.

The other hymenoptera show no difference in principle. Where parthenogenesis results in the formation of both males and females, the former develop with the haploid number of chro-

[1] For cytological facts see Nachtsheim, H. ('13).

mosomes (case *2A*), the latter with the diploid number (*2B*). Spermatogenesis and fertilization are the same as in the bee (*Neuroterus* according to Doncaster, '10). If parthenogenesis results in female offspring, development occurs with the diploid number, no reduction taking place in spite of two maturation divisions (*Nematus*, Doncaster, '09, *Rhodites*, Schleip, '09). Occasional males as in *2A* or *2Cb*.

2. *Rotatoria.*—The relation of parthenogenesis to sex seems to be exactly the same as it is in Hymenoptera. (Lauterborn, '98, Shull, '10, Whitney, '09). Parthenogenetic eggs without reduction give females, with reduction males, the latter if fertilized, females. We must suppose that the spermatogenesis is similar to that of the bee.

3. *Aphids.*—The well-known work of Morgan ('09) and von Baehr ('09) shows them to fall into case *B*.

4. *Phasmids.*—Their behavior is not yet clear, either experimentally or cytologically. Probably they behave like some gall-wasps with occasional males (see von Baehr, '07).

5. *Lepidoptera.*—The group of the Psychidæ, which exhibits regular parthenogenesis, is cytologically most interesting, as will be shown in a paper by Dr. Seiler now in press. The results seem to fit our conceptions. About the cases of facultative parthenogenesis as described here we know only that both sexes are produced and contain the diploid number of chromosomes. We must suppose that we are concerned with case *1Cb* or *1Cb* combined with *1B* or *1Ca*.

6. *Ostracoda and Cladocera.*—Although these groups are greatly favored by experimentalists, we know comparatively little about their cytology (Woltereck, '98, Schleip, '09, Kuehn, '08). It is possible that they belong to the same group as the Rotatoria, but in *Ostracoda* parthenogenetic female-producing eggs undergo no reduction division. The experimental results in sex-production in this group make it seem possible, however, that we are here concerned with something quite different. This possibility will be discussed on another occasion.

7. *Artemia.*—The most recent writers on the subject (Artom, '11, Fries, '09) agree that the parthenogenetic races develop with the diploid number of chromosomes, the bisexual races in

the usual way. Nothing is known about the cytology of the occasional males in the parthenogenetic races. It is therefore impossible to tell with which of the possibilities we are here dealing.

8. *Nematodes.*—The strange type of parthenogenesis described for a Rhabditis by Krueger ('12) and resulting in female offspring, occurs without a reduction of the chromosomes. Here we have case *2B*.

9. *Echinoderms.*—(Artificial parthenogenesis.) Tennant's work makes it pretty certain that the male is heterozygous. Artificial parthenogenesis with reduced chromosome number ought to yield males as in the bee. This has been found to occur in the few recorded specimens.

10. *Amphibia.*—(Artificial parthenogenesis.) We do not know which sex is heterozygous in Amphibia. R. Hertwig thinks it is the male. We tried ('11, '13) to show that the experimental results favor the view of female heterozygosis. Moreover we do not know whether parthenogenesis takes place with the haploid or the diploid number of chromosomes. The expectations can therefore fit any of the above enumerated cases.

I want to emphasize, finally, the fact that our thesis, if expressed in terms of cytology, is nothing but Wilson's old hypothesis of the one portion-two portion *x*-substance. But our experiments have allowed us to give a physiological meaning to this conception and to bring this cytological conception into harmony with Mendelian formulations.

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BIOLOGICAL BULLETIN

REACTIONS OF AMEBA TO LIGHT AND THE EFFECT OF LIGHT ON FEEDING.

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CONTENTS.

INTRODUCTION.

The reactions of ameba-like organisms to light have been studied by a number of investigators in order to determine whether "naked" protoplasm is capable of responding to light waves. Whenever intense light was used as a source, amebas, pelomyxas and plasmodia reacted negatively, and as a consequence it was tacitly assumed that in general naked, "undifferentiated," protoplasm reacted negatively to light.

But speaking now only of experiments performed on amebas, for I do not wish to leave the impression that in my opinion what is true of the behavior of amebas toward light holds also for

plasmodia and pelomyxas, the first to be recorded appear to be those of Verworn ('89). He projected white light and various spectral colors of various intensities perpendicularly through the microscope slide, and observed no change of reaction as the ameba moved from one intensity to the other, or from one color to the other. With the experiment similarly staged, Davenport ('97) came to the same conclusions as Verworn. Amebas moved from a field of very weak light into one of very strong light, apparently without change of behavior, even when the change of intensity was sharp and abrupt. But Davenport showed that when a light beam is projected horizontally against an ameba, the ameba orients so as to flow away from the source of light. Harrington and Leaming ('00) showed that intense white, violet, or blue light, flashed on a moving ameba, arrested its movement momentarily; but red light was without any decisive effect. Mast ('10), experimenting under conditions similar to those under which Verworn and Davenport worked, confirmed Davenport's findings when horizontal beams of light are thrown against an ameba, and also concluded from his experiments that when an ameba finds a perpendicular beam of intense light in its path, it avoids the light in many cases. Mast also confirmed the general conclusions of Harrington and Leaming.

None of these investigators observed any but negative behavior, though Mast presumably looked for positive responses, for he says "I was unable to obtain positive reactions in *Stentor cæruleus*, *Amœba*, and fly larvæ" ('11, p. 270). The reason Mast failed to get positive responses was because the beams of light which he used were too large and the light was too intense. And further his apparatus was perhaps defective. He says: "The beam of light was produced by focusing a limited area of a luminous Welsbach mantle on the slide by means of *the mirror* and an Abbe condenser" ('11, p. 78). If he used an ordinary microscope mirror, as he seems to have, a very faint subsidiary beam as well as the very intense primary beam, was projected through the slide. The one is produced, of course, by the front surface of the glass, and is very readily overlooked; the other is produced by the surface of the silvering. Since the subsidiary image shows only on one or two sides of the main image, if square,

the chances are about even that the ameba came into contact with the subsidiary image first, and therefore the likelihood of a positive response is increased. From my own experience I have found that whenever mirrors are necessary, it is absolutely essential that they are silvered on the front surface; otherwise subsidiary images will result, and in case monochromatic spectral light is used a considerable degree of impurity may occur. If Mast used back surface mirrors in his work on the light reactions of ameba, some of his experimental results are, therefore, incompletely described and consequently inconclusive; and his inability to observe positive responses was due, in part, to improper staging of the experiment for this purpose.

The great majority of the light experiments in this paper show positive reactions; a few are indifferent, and a few negative. This proportion resulted from the manner in which the experiments were set, and from the fact that observations were based upon the behavior of amebas *before* they came into contact with the beam of light as seen by the eye, instead of after, as previous observers did. But in a great many cases negative behavior did not result even after the ameba came into contact with the light; but if the beams had been larger it is not improbable that the proportion of negative reactions would have been larger.

These experiments on the reactions of ameba to light were performed not only for the purpose of testing the sensitiveness of these organisms to light by itself, but especially to see whether differences in intensity, quality or direction of light rays are capable of causing changes in the behavior of amebas while feeding. In order to let the results speak as definitely as possible, a number of experiments were performed first with beams of light only as stimuli. Later, particles of food were presented in connection with the beams of light. By comparing these two sets of experiments with each other and with the results of previous experiments on the feeding habits of ameba (see Bibliography for references) the effect of light on feeding may be readily observed.

Several investigators, as already pointed out, made observations on the reactions of ameba to light, but all of them made use of large areas of very intense light. But for purposes of

comparison with other sources of stimuli it was thought essential, in my own work, to reduce the areas of light to a size comparable with the size of food and other objects which were placed at the disposal of the ameba. By throwing the beams of light vertically through the slide on which the amebas were placed, the area of the cross section of the beam of light used for stimulating the ameba could be varied within the desired limits.

As sources of light the Welsbach gas mantle and the Leitz Lilliput arc were used. The gas light was passed through five cm. of distilled water containing a very small quantity of an ammoniacal solution of copper sulphate to absorb the excess of green and yellow rays and the distinctive heat rays. Between the water and the microscope was interposed an opaque screen with several small, clear-cut pinholes in it. By moving the screen away from or toward the microscope, and focusing with the substage condenser, the size of the projected beams of light through the microscope could be easily controlled. The ordinary mirror of the microscope was discarded and a front surface mirror placed in its stead, in order to avoid reflection from the front surface of the glass and so producing a subsidiary image on the slide. The great sensitiveness of ameba to light makes this precaution absolutely necessary.

When spectral light was used, the arc was employed as a source, and either prism or grating interposed between clear distilled water and the screen.

The amebas were placed on large, clear thin cover glasses in clear culture fluid, without a cover glass over them. Usually in these light experiments the beams of light were left stationary while the coverglass containing the ameba was shifted, whenever shifting was necessary for experimental purposes. Both intermittent and continuous beams of light were employed. Intermittent light was produced by moving an opaque object up and down between the screen and the microscope. In a general way, continuous and intermittent light had about the same effect on the ameba. After orientation, it is worth noticing, the ameba advanced as definitely and as uniformly toward intermittent as toward continuous light.

The work was done in a dark room in which there was very

little diffuse light. Some light was necessary in order to make the camera lucida drawings illustrating the experiments. But this light was confined, as far as possible, to the paper on which the drawings were made, and no more light was used than was necessary. Extra precautions were taken to prevent any but vertical beams from reaching the ameba.

Experiments with Light.

White Light.—Two beams of white gas light were projected in front and to the right of an *Amœba dubia* flowing along in spatulate form—Fig. 1. The ameba flowed straight past the small spot of light and also passed the larger beam a short distance, when two side pseudopods were thrown out directly toward the larger beam. The pseudopod nearer the light spot enlarged until it had flowed over the light, then it was arrested and the other pseudopod became the main one through which the ameba moved away. The slide was then moved so that the small beam lay in front of the ameba—Fig. 5. Two pseudopods were formed on the left side. The main pseudopod moved into contact with the small beam of light, then sent out on the left a pseudopod which moved directly into contact with the larger beam. At the same time the more posterior of the previously formed pseudopods sent out on its right a pseudopod which also moved into contact with the larger beam while the more anterior pseudopod was withdrawn, but this pseudopod was finally withdrawn as the one which previously moved into contact with the light, moved on over the light spot—8. These experiments show very clearly that small beams of white light attract amebas before they actually come into contact with the beams.

An *Amœba proteus*[1] was placed so that a small beam of white light lay to the right of its path—10. Two pseudopods were thrown out, one on the left and one on the right, but both were quickly retracted. The tip of the ameba then turned sharply to the right and directly toward the light—14. The ameba then moved forward in the original direction for a short distance

[1] The name *Amœba proteus* of Pallas and Leidy as used in this paper also includes the species *A. discoides* Schaeffer, discovered to be distinct from *A. proteus* after the experimental work recorded in this paper was done (for preliminary descriptions see Schaeffer, '16*b*).

—16. Finally a pseudopod was thrown out on the right partly encircling the light—19. After breaking up into a number of pseudopods the ameba moved away. The ameba was shifted with the beam of light on the left—22. Movement became somewhat uncertain at first, then streaming was reversed—25. As the ameba moved forward in the new direction, it gradually turned to the right until it was flowing directly toward the beam of light—29. After partly surrounding the beam—35—the ameba moved away.

Summary.—From the foregoing experiments it is clear that ameba responds positively to white light under the given conditions. The ameba is attracted from a distance of sixty-five microns or more. Since the beam is projected vertically, the question at once arises: How does the ameba become aware of the beam of light? Is some light reflected horizontally by particles of solid matter in the water; or is some of the light energy transformed into heat or other form of energy which, being radially propagated, stimulates the ameba? These are very important questions in sense perception, but they must remain unanswered for the present. The light apparatus at my disposal was too crude to attempt a solution of them.

In most cases the ameba reacts positively until it comes into contact with the beam of light, when negative behavior usually sets in. This difference in behavior may be due to differences in the intensity of the stimulus. Both *proteus* and *dubia* react positively to white light.

It is quite clear from these experiments that light of the intensity used does not tend to inhibit directly the formation of pseudopods, nor does it seem to have any other direct effect on the movement or form of ameba.

Red Light.—The apparatus for producing monochromatic light of the various wave-lengths was a Leitz Lilliput arc light, fifteen cm. of distilled water, a piece of grating with a slit two millimeters wide, a screen of heavy drawing paper with a pinhole about one half mm. in diameter with clear-cut blackened sides, front surface mirror and condenser. The screen was blackened on the far side and lighted on the near side with just enough light from a Welsbach mantle to barely see the amebas. The spectrum on

the screen was about two cm. long. By means of the pinhole therefore only about one fortieth of its length was projected at any one time. The apparatus was rather crude and somewhat unsatisfactory, but it seemed to be sufficiently reliable for exploratory work.

On account of the impracticability of testing spectroscopically the beams of light which were thrown into the microscope, no accurate values in wave lengths can be given for blue, red, yellow, etc., so there may have been, for example, a few "rays" of orange or green in the yellow when that color is specified; but such mixtures cannot have been very significant since the size of the pencil of light was very small, roughly one fortieth of the length of the visible spectrum. As a matter of fact some rays of all wavelengths were mixed with the pencil of monochromatic light, but this defect could not be remedied, for some diffuse light (though this of course might be monochromatic spectral light) is necessary in order to see what is going on. But if any change in behavior takes place with reference to a pencil of monochromatic spectral light, the change must be caused by the more intense monochomatic light, since the diffused white light is equally intense all over the field.

A small beam of red spectral light was projected in the path of a *proteus*—37. The ameba moved forward a short distance, then sent out a pseudopod on the left—40. From this pseudopod another was sent out on the right—45—which moved directly toward and over the red beam. The pseudopod was soon withdrawn and the ameba then moved on.

Another *proteus* was brought into view with the light on the right—49. The ameba turned sharply to the right, then to the left, and finally moved over the red light. Presently a pseudopod was thrown out on the right through which the ameba moved away.

In the next experiment the light was placed on the left of a *proteus*—53. The ameba moved forward for a short distance, then threw out a pseudopod on the left—56—through which the ameba moved off without coming into contact with the light. The ameba was then shifted with the red beam lying on the left —59. As the ameba flowed forward toward the light spot a

pseudopod was thrown out on the right, through which the ameba finally moved off. The ameba was then shifted so that the light lay on the right—63. The ameba turned to the left and moved on. The ameba was again shifted with the light lying on the left—65. As the ameba moved forward a small pseudopod which was thrown out on the left moved directly into contact with the light. The next four experiments—69–93—all show that the beam of light was sensed before the ameba came into contact with it, and also that the light produced more or less definite positive behavior, followed by indifferent or negative behavior.

A beam of red light was projected in the path of another *proteus*—94. A pseudopod was thrown out toward the light. After it came into contact with the light—97—the ameba moved away through a pseudopod thrown out on the left—99. The ameba was then shifted with the beam of light on the left —101. The ameba moved forward, then turned slightly to the right—104. A small pseudopod was then sent out toward the red light—107—but after covering half the distance it was retracted while a pseudopod was thrown out on the same side, but further forward. From this pseudopod still another was sent out on the left. The ameba thus partly encircled the red light. The ameba was shifted again with the red light lying in front of it—111. The ameba threw out a pseudopod on the right and passed on—111–113—but when the tip of the main pseudopod extended beyond the light, it broke up into four pseudopods—115—of which the left posterior ultimately became the main pseudopod through which the ameba moved away.

Another *proteus* was then brought into the field with a beam of red light lying to the right—120. The ameba moved on a short distance, then threw out two pseudopods on the right—122 —one of which moved into contact directly with the red light —125—but which was retracted as the ameba moved on. The ameba was then shifted with the light lying on the left—125. Pseudopods were sent out on both sides but the one on the right finally became the main one leading the ameba away. The ameba was again shifted with the red light lying on the right—128. A pseudopod was thrown out on the right directly toward

the light. When it came into contact with the light it forked, the limbs moving forward with the light spot between them —133. The right limb became the main pseudopod through which the ameba moved off.

A beam of red light was projected to the right of another *proteus*—167. The tip of the ameba turned slightly toward the left, then resumed its original direction. The ameba was then shifted with the red light on the left—170. There was at first a tendency for pseudopods to form on the right, but, as the ameba moved forward, two were formed on the left in the region of the light. One of them moved a considerable distance toward the light—174—but was then retracted as a pseudopod on the right was thrown out to become eventually the main pseudopod. The ameba was then shifted with the red light lying on the left—176. As the ameba moved forward a large pseudopod was thrown out on the left, directly toward the light. This pseudopod became the main one through which the ameba moved away.

Summary.—Red spectral light produces about the same changes in behavior as white light. The vertical beam of red light is sensed at a distance, and in almost all cases produces positive behavior. In some few cases an ameba may behave indifferently or even negatively; but if the experiments are repeated several times a positive reaction is almost sure to occur. Amebas are therefore not negative or positive permanently with respect to beams of red light, but the behavior may readily change from the one to the other aspect. Red light of the intensity used does not seem to stimulate the ameba disagreeably when it moves into direct contact with the beam of light, for in a number of cases the ameba moved on over the light without visible change of behavior. In some respects the ameba tended to encircle the source of light in the same manner in which it sometimes encircles solid objects.

Blue Light.—A beam of impure[1] blue spectral light was projected to the right of a *proteus*—135. The ameba turned to the

[1] Between the grating and the arc was placed twenty cm. of distilled water containing a very little ammoniacal copper sulphate. The copper salt gave rise to a subsidiary faint yellowish spot of light not quite coinciding with the blue. The yellow image disappeared when the copper salt was omitted from the distilled water. The yellow image was probably due to fluorescence of the copper sulphate.

left and moved away, a decided negative reaction. The light was shifted so that it lay directly ahead of the ameba—139. The ameba threw out three pseudopods on the left, but almost immediately retracted them. Another was sent out on the right, but it also was soon retracted. The ameba then moved forward, and in passing the blue light sent out a little pseudopod toward it—143. The ameba finally turned to the left and moved on. The ameba was shifted again with the blue light on the right—145. After the tip of the ameba had passed by the blue light it turned to the right. A small pseudopod was also sent out toward the light—148. The ameba was then shifted with the blue light lying directly in front—150. As the ameba moved forward a small pseudopod was thrown out into contact with the light—152. Then the ameba moved on. The ameba was shifted again with the blue light directly ahead—154—but a decided negative reaction set in. But when shifted again with the blue light ahead—158—the tip of the ameba turned away from, and then toward the light, but finally moved on in the original direction. The ameba was shifted again with the blue light directly ahead—162. The resulting behavior was indefinite.

Another *proteus* was then brought into the field with a beam of blue light directly ahead—181. The ameba then threw out a pseudopod on the left and from this one another on the right, and from this last one still another on the right, so that the light was partially encircled. The ameba then moved off through a pseudopod on the left. The ameba was shifted with the blue light on the right—186. A pseudopod was thrown out on the right directly toward, and into contact with, the beam of light. The ameba then flowed away through another pseudopod on the right. The ameba was again shifted with the light lying on the right—190. Two pseudopods appeared on the right, one of which was directed toward the light spot. Both pseudopods were presently retracted and the ameba moved on to the left. Very peculiar behavior was observed when the ameba was shifted again—194. The behavior was at first negative, the ameba moving away to the right—195—but streaming was then reversed, and as the ameba passed by the light, two small pseudopods were sent out toward it—200. They were retracted however

as the ameba moved on. When shifted again with the blue light to the left—201—the ameba turned toward the light and then passed on to the left. The ameba again reacted positively when shifted with the light straight ahead—204. When shifted again the ameba reacted positively but rather uncertainly—209. In the next experiment, with the beam of blue light on the left, negative behavior was induced—216.

A beam of pure (see footnote p. 11) blue spectral light was projected to the right of a *dubia*—219. The ameba moved past the light spot for a considerable distance without any change in behavior. Then two pseudopods were sent out: one directly toward the light, and the other near the tip, but also on the right side—220. As the pseudopods enlarged, the tip of the ameba also turned sharply to the right—221. When the posterior pseudopod came into contact with the light, the pseudopods on the right were retracted, and two others thrown out on the left —222—but these also were retracted after a few seconds, and the ameba then moved on in the original direction. The ameba was then shifted with the blue light lying directly ahead—223. The tip of the ameba (only the tip of the ameba is shown) turned to the left—224—but a pseudopod was thrown out on the right toward and into contact with the light—225. The ameba flowed partly over the light—226—but withdrew from it later and moved off through a pseudopod on the right.

Summary.—There is no marked difference between the reactions toward red light and those toward blue. Blue light induces positive behavior in as marked a degree as red, though when all the experiments are considered, red light seems to be somewhat more attractive than blue. Blue light, like red and white, induces both negative and positive reactions. Blue light can also be sensed at a distance.

The experiments with the *dubia*—219–222—are interesting inasmuch as a pseudopod was thrown out at the tip of the ameba on the side on which the light lay, some time after this part of the ameba had passed the light. It may be noted also that the tip of the ameba turned strongly in the same direction. It appears quite unlikely that the light acted as an efficient cause on this region of the ameba at the time of the formation of the pseudopod,

for the effect of the light was continually decreasing in intensity as the ameba moved away from it. The light would therefore be expected to have the maximum effect at maximum intensity, which was when the tip of the ameba was closest to the light. It is improbable therefore that the throwing out of a pseudopod and the bending of the tip to the right were caused by the impinging of the light rays at that region at that time. It is possible that this behavior is the result of the cumulative effect of the light rays while the ameba was passing the beam. There was formed a tendency toward a positive reaction some few seconds before it expressed itself in visible change of behavior, and when this tendency "came to a head" it resulted in exaggerated behavior; for two pseudopods were thrown out on the stimulated side and at the same time the tip of the ameba was turned to the right. This feature of ameban behavior—the formation of two pseudopods on the stimulated side, one near the anterior end and the other opposite the stimulating object after the tip of the ameba has passed the stimulating object—is frequently observed and is of great interest. It indicates several things. First, it effectively disposes of the hypothesis that the movement of pseudopods toward an object is directly induced by the object. Second, it shows that there is some sort of a coördinating or integrating agency at work in the ameba so that the larger part of it, at least, tends to react in a coördinated manner, even if there are separate centers of reaction. When the posterior pseudopod came into contact with the light, negative behavior set in suddenly. The pseudopods on the right were promptly withdrawn and two others were rapidly projected on the left. Nevertheless, the ameba finally moved on in the original direction.

Violet Light.—The violet light that was selected was as near to the end of the visible spectrum as possible. A beam of violet light was projected on the right of a *proteus*—230. A small pseudopod was thrown out on either side—232—the one on the right being directed toward the light. The ameba moved away however through the pseudopod on the left. The ameba was shifted with the light again on the right—235. The tip of the main pseudopod turned to the right and moved into contact with and then on over the light. The ameba was shifted with the

violet light on the left—241. As the ameba moved forward past the light a small pseudopod was sent out toward the light, but it was withdrawn before it came into contact with the light.

When shifted again with the light on the right—245—the ameba sent out a pseudopod anterior to the light—249—but it curved backwards toward the light as the ameba moved forward—251. On the next trial—253—the ameba first turned away from the light then sent out a pseudopod directly into contact with it; then another pseudopod was sent out on the side and anterior to this one.

Summary.—Amebas react positively, negatively, or indifferently toward violet light. The greater number of changes of behavior produced by violet light were positive. No definite differences could be observed between the effects of violet light and those of any other spectral light thus far described.

Green Light.—A beam of green spectral light was projected to the right of an *Amœba dubia*—259. As the ameba moved forward the tip of the main pseudopod moved to the left. A small pseudopod was formed on the right toward the light. The ameba then turned toward the right and at the same time threw out a pseudopod on the right near the tip of the main pseudopod. Both pseudopods were withdrawn as the ameba moved on. (Compare the behavior of this ameba with that illustrated in Figs. 219–222.)

The beam of green light was then projected to the right of a *proteus*—262–265. As the ameba moved forward, a large pseudopod which was thrown out on the left, was soon retracted, the ameba moving on in a straight path. The ameba was shifted—266–270—with the green light to the right. As the ameba moved on past the light, a small pseudopod appeared on the right near the light, but it was retracted before it had developed to any extent, as the ameba flowed on.

These few experiments indicate that the effect of green spectral light is similar in a general way to that of white, red, blue, etc. Although the positive reactions in these experiments are slight, they are nevertheless definitely positive. If I had made as many experiments with green light as with red or blue, I have no doubt that more decided reactions would have been obtained.

Yellow Light.—A beam of yellow spectral light was projected straight ahead of a *proteus*—271. The ameba moved on without any definite change of behavior and passed over the beam of light. The change in the direction of movement—275–277—indicates that the yellow beam had a disagreeable effect after the ameba came into contact with it. When the ameba was shifted—278—the tip of the main pseudopod turned to the left—a negative reaction continued from the previous experiment. But while passing the beam of light the negative condition gave way to a positive as is shown by the turning of the tip of the ameba toward the light—282. A pseudopod was then thrown out on the left on the convex side, and from this one another on the left through which the ameba moved on, again a negative reaction. The ameba was shifted again—285—with the yellow light ahead. The ameba turned sharply to the right, but as it passed by the light a pseudopod was thrown out on the left directly toward the light—287. This pseudopod became the main one through which the ameba flowed on over the light. As the ameba came nearly into contact with the light, a pseudopod was thrown out on the right—290—an indication of a negative reaction, but it was soon retracted.

To summarize: Amebas respond positively, negatively or indifferently to beams of yellow spectral light. As far as my experiments go, yellow light has about the same effect as red or blue or the other spectral colors which have so far been considered.

Orange Light.—A beam of orange light was projected to the left of a *proteus*—294. The ameba turned to the left and moved directly into contact with the light. When the ameba came into contact with the light, a pseudopod was started on the right, but it was soon retracted and the ameba flowed on over the light without further change of behavior. The ameba was then shifted with the orange light on the left—301. The tip of the ameba turned to the left, then broke up into two pseudopods of which the left one turned still further to the left and finally became the main pseudopod through which the ameba flowed away. The ameba was shifted again with the orange light slightly to the right—306. A pseudopod which was thrown out on the right elongated as it turned to the left. When the tip of the pseudo-

pod had passed the light, a new pseudopod was thrown out on the left near the light—309. This pseudopod moved straight forward for some distance, when another pseudopod was sent out on the left—312—but this one was finally retracted as the ameba moved away.

Orange spectral light induced positive reactions in the ameba of this series of experiments, though they were wholly positive only in the first experiment. In the other experiments the tendency was toward positive behavior, but the source of stimulation was not definitely sought. The beam of light attracted the ameba only mildly after the first encounter, and the tendency to move forward (Schaeffer, '14*a*) may be presumed to have been about as strong as the tendency to move toward the beam, hence the partial encircling of the beam in the last two experiments. In my laboratory notes there is recorded one experiment with orange light in which the behavior was wholly negative.

Experiments with Dark Beams.

When it was seen that white light and spectral light of various wave-lengths had essentially the same effect on ameba, it seemed likely that these results were due to differences in intensity between the beam of light and the diffuse light on the field. The suggestion then presented itself whether a decrease in intensity of light in a small area produces a similar result. A dark beam was therefore projected into the microscope. The source of the dark beam was a hole in the screen, leading into a blackened light tight box fastened to the back of the screen. The sides of the hole were blackened to prevent as far as possible the reflection of light. The rest of the screen was illuminated by diffuse light, as in the other experiments, but more brightly so as to increase the contrast between the field and the hole. The hole as viewed through the microscope appeared as a very dark gray spot.

A *proteus* was shifted so that the dark spot lay directly ahead of the ameba—314. The tip of the ameba broke up into two pseudopods, one of which turned to the right and the other to the left of the dark spot, indicating a negative reaction. As the right pseudopod moved forward it turned to the left until it

came into contact with the dark spot—a positive reaction. The ameba then moved on through this pseudopod and partly over the dark spot. The ameba was again shifted so that the dark beam lay directly ahead—319. A pseudopod which was thrown out toward the right led the ameba away, a definite negative reaction.

Another *proteus* was then brought into the field with the dark spot directly ahead—322. The ameba turned to the right and moved on, avoiding the dark beam. The ameba was then shifted with the dark spot straight ahead—325. The ameba became irregular in its streaming at the anterior end, indicating that the ameba sensed the dark spot and that there was present a tendency to react negatively; but the tendency to negative reaction was weak, for the ameba started presently to move over the dark area. The ameba, in irregular shape, was shifted again with the dark spot directly ahead—329. When the ameba came into contact with the outer edge, the tip of the main pseudopod forked, the right prong becoming the main pseudopod through which the ameba moved away. Negative behavior is again shown here. The ameba was shifted with the dark spot directly ahead—333. A pseudopod was thrown out on the left as the ameba moved into contact with the dark spot—334—indicating a tendency to negative reaction, but it was withdrawn as the tip of the ameba proceeded for some distance beyond the further edge of the dark area—336. The ameba was moved again so that the dark spot lay slightly to the right—340. The ameba moved into contact with the dark area, then sent out a pseudopod on the left, but it was soon withdrawn and at the same time another was sent out on the right. The ameba finally moved on in the original direction. Here we have first positive behavior in the turning of the ameba toward the dark spot; then negative behavior in the formation of the pseudopod on the left; then again positive behavior in the resumption of forward movement and the formation of the pseudopod on the right. In the next trial the ameba was moved with the dark spot slightly to the right—346. The ameba turned slightly further to the left, then directly toward the right and toward the dark spot—348. When the ameba came into contact with the dark spot, a pseudopod was thrown out on the left, but it was withdrawn as the ameba moved on.

Another *proteus* was then brought into the field with the dark spot slightly to the left—354. The tip of the main pseudopod broke up into two pseudopods of which one moved directly toward and over the dark area. But when the ameba came into contact with the dark spot, a large pseudopod was thrown out on the right—356—but it was withdrawn as the ameba moved on over the dark spot. The ameba reacted in effect positively throughout the experiment, but a strong tendency to react negatively is shown by the breaking up of the main pseudopod into two pseudopods—355—and by the appearance of the pseudopod on the right when the ameba came into contact with the dark spot —358. The ameba was then shifted with the dark spot slightly to the left—359. The ameba moved forward a short distance, then the tip of the main pseudopod spread out, and then the protoplasmic stream was suddenly reversed and the ameba moved away to the right through a vestige of a previous pseudopod—a decided negative reaction. But the ameba was then moved with the dark spot directly ahead—363. After the ameba had moved forward a short distance the tip forked broadly, and the ameba moved off through the left prong, again a decided negative reaction.

Summary.—Amebas become aware of dark spots before they come into contact with them, as seen through the microscope with the eye, just as they become aware of beams of light before encountering them. In most cases the tendency is to react negatively, but in some instances the first change in behavior is positive. Usually when the ameba first comes into contact with the dark beam there is a tendency toward negative behavior, as is shown by the formation of pseudopods which, if they became main pseudopods, would lead away from the dark area. These pseudopods are usually withdrawn as the ameba moves forward over the dark spot. The behavior is seldom wholly positive or wholly negative; in most cases there is some vacillation between negative and positive reactions. The reactions on the whole were not so pronounced as those toward light. The actual stimulating quality is very likely to be looked for in the difference in light intensity between the dark spot and the field.

Reactions toward Solid Particles when Stimulated at the Same Time by Beams of Light or of Darkness.

A grain of globulin was placed over a small beam of blue spectral light, and arranged so that the illuminated globulin lay in the path of an *Amœba proteus*—365. The ameba moved in spatulate form directly toward the globulin-blue light until it came into contact with the globulin, when a pseudopod appeared on the right. The ameba however ingested the globulin in typical manner and then quieted down over the blue light for over twelve minutes.

A grain of globulin was placed in the path of a *proteus* with a beam of green spectral light between the ameba and the globulin —373. Through a pseudopod thrown out on the right the ameba moved away from the light-globulin—374. The ameba then broke up into four pseudopods of which the left one of the middle pair became the main pseudopod. The ameba moved forward through this pseudopod toward the globulin in a curved path, apparently, so as to avoid the light, pushed the globulin ahead a short distance, and then ingested it in an imperfect food cup. This is an interesting experiment. The beam of green light stimulated the ameba negatively when contrasted with the globulin. The ameba made a detour around the light to get to the globulin. This experiment should be compared with Figs. 1–13 in a recent paper (Schaeffer, '17*b*) in which very similar behavior is recorded as an ameba moved toward a grain of globulin with a grain of silicic acid lying immediately in front of the globulin.

A grain of globulin was placed to the left of a *proteus* with a beam of green spectral light between the globulin and the ameba —386. The ameba moved forward a short distance, then bifurcated, the right prong being directed backwards while the left prong was directed toward the globulin—388. The ameba moved toward the light at first—389—but presently the tip of the ameba broke up into two pseudopods. The one thrown out on the right enlarged rapidly as it moved in a slight detour around the light toward the globulin—392, 393. After rolling the globulin along the surface for a short distance it was ingested in a typical food cup. This experiment as well as the preceding,

shows that a beam of green light acts as a disturbing factor when an ameba is stimulated at the same time by globulin.

A grain of globulin and a beam of yellow spectral light were placed to the right of the path of a *proteus* with the light between the globulin and the ameba—397. As the ameba moved forward it turned to the right and directly toward the yellow light. The ameba moved over the light, then turned to the left and moved into contact with the globulin, which the ameba rolled around a short distance before ingesting it in a normal food cup. The yellow light did not disturb the ameba when stimulated simultaneously by globulin.

A grain of globulin and a beam of yellow light arranged as in the preceding experiment were placed in the path of another *proteus* —408—but the behavior observed was negative, due doubtless to lack of hunger in the ameba. The ameba was in Y-shape at the beginning of the experiment—408. The ameba responded negatively by bending the right prong to the right and flowing along it. A pod was thrown out on the right, indicating the presence of a tendency to a positive reaction. The ameba was then shifted with the yellow beam straight ahead and the globulin a little to the left—412. The tip of the ameba forked, the axes of the limbs coinciding with the same straight line, and nearly perpendicular to the rest of the ameba—413. The right prong turned toward the yellow light—415—and presently two pseudopods were sent out a short distance toward the globulin—416 —but both were withdrawn as the ameba moved away through a pseudopod thrown out on the right—417. The ameba was shifted with the globulin straight ahead and the beam of yellow light to the left—419. The ameba moved forward a short distance when a pseudopod was thrown out to the left—421. This pseudopod became the main one, and after it flowed ahead some distance it turned to the right and moved toward the globulin —423. The tip of the ameba then turned to the right still more strongly and at the same time a pseudopod was thrown out in the direction of the stimulating objects—424. The posterior end now became activated but only for a short time—425. Several new pseudopods were formed indicating uncertainty in behavior, of which the one on the right extending toward the globulin

became for the moment the main pseudopod—427. After moving toward the globulin for a short distance a pseudopod was thrown out on the right leading the ameba away from the test objects—429-432. Presently however the main pseudopod bent strongly to the left—433, 434—with the formation at the same time of two pseudopods on the convex side of the main pseudopod—434, 435. The pseudopod pointing toward the globulin moved forward a short distance—436—but this pseudopod was retracted as the other one of the two formed on the convex side became reactivated leading the ameba away from the globulin and light.

Since it was evident that the ameba reacted negatively to both light and globulin when these substances stimulated the ameba at the same time, two further tests were made on this ameba in which each test substance was used by itself.

The piece of globulin was laid some distance ahead of the ameba —438. The ameba moved toward the globulin a short distance then threw out a pseudopod on the left—440—through which the ameba moved forward with the globulin on the right—442. A pseudopod was then thrown out on the right toward the globulin while the tip of the main pseudopod turned strongly toward the left—444. When the pseudopod on the right came into contact with the globulin the main pseudopod was retracted —447, 448. The globulin was only partly surrounded—449. One of several pseudopods formed on the right led the ameba away, leaving the globulin behind.

After a few minutes the beam of yellow light was projected in front of this ameba—453. As the ameba moved forward several pseudopods were thrown out on either side of the main pseudopod, giving the ameba a very irregular shape. When the ameba came nearer the light—458—it advanced definitely forward passing the beam immediately to the right—459. The main pseudopod then swerved a little to the side as two pseudopods were formed on the right, through the lower one of which the ameba finally moved away from the light.

This ameba then reacted definitely positively to globulin but much less definitely positively to yellow light, when presented separately, but decidedly negatively when presented together.

The experiment shows that yellow light was the cause of the negative behavior when both it and globulin were presented together; or perhaps one ought to say yellow light, *in combination*, possessed deterring qualities which were absent when it stimulated the ameba alone. Here again we have another case of where the milder of two positively stimulating objects when encountered alone becomes negative when encountered simultaneously with the more strongly stimulating object (Schaeffer, '17*b*).

A dark spot with a piece of carbon lying in it was then placed in the path of a *proteus*—463. As the ameba moved forward it turned slightly to the left at first—467—but the tip of the ameba then turned to the right and moved directly toward the dark beam and carbon—471—until within about ten microns of the beam, when the ameba suddenly turned to the right—472. The ameba moved along in this direction without coming into contact with either the dark spot or the carbon. That this decided negative reaction was caused by the dark spot is evidenced by the next experiment in which the carbon was omitted—481. The ameba moved forward toward the dark beam for a short distance, then became very uncertain in its behavior. First it turned to the right, then formed a pseudopod on the left through which it moved forward with the dark spot on the right—484. A pseudopod was thrown out on the left but it was promptly retracted—487. At the same time the tip of the pseudopod turned very sharply to the right and moved directly toward the dark spot—488. The ameba continued moving toward the dark spot until within thirty microns of it—489—when the main pseudopod was retracted and the ameba then moved away to the left through another pseudopod which had been slowly forming while the ameba was moving toward the dark beam. A dark beam with a piece of globulin lying in its centre was placed to the right of the ameba with a pseudopod already turned toward the dark spot—492. The ameba flowed on through the pseudopod directly toward the dark spot. When within about eighty microns of it, the tip of the main pseudopod forked—495—indicating a tendency to negative behavior. The left prong which became the main pseudopod, moved directly

forward until it came nearly into contact with the dark beam when a slender pseudopod moved into the dark area, and another, indicating a tendency to negative behavior, moved to the right —498. But when the ameba came into contact with the globulin the pseudopod on the right was withdrawn and then the globulin was ingested, the ameba quieting down over the dark spot. The experiments with this ameba show that the dark beam may be sensed at a distance of at least 150 microns, and that the globulin may be sensed as well apparently when lying on a dark spot as when illuminated.

A dark spot with a fragment of carbon lying on it was placed to the right of an ameba—503. There was no definite response. The ameba turned toward the right and moved toward the test objects in a more or less uncertain manner—504, 505. A pseudopod was then sent out on the left—506. After it had attained to considerable size it was withdrawn and the ameba moved off through a pseudopod thrown out on the right—508. The ameba was shifted with the carbon-dark spot lying on the right—509. As the ameba moved forward it turned toward the right—510—a small pseudopod being formed, as frequently happens, on the convex side, but a pseudopod was presently thrown out on the left—513—through which the ameba moved away. To show that it was the dark spot and not the carbon which produced the negative behavior, the results of the next experiment are appended—514. The ameba moved directly into contact with and on over the carbon without any sign of a negative reaction. A grain of globulin lying in a dark beam was then placed at some distance to the right of the ameba—520. An accidental jar displaced the globulin so that it lay near the anterior edge of the dark spot. The ameba changed its direction of motion and moved straight toward the globulin and the dark beam—521. (It may reasonably be doubted whether the ameba sensed the globulin at this distance.) The globulin was then moved to the further side of the dark beam—522. The ameba kept on moving forward for some distance, then its behavior became somewhat uncertain. The ameba moved slightly to the left—524. A pseudopod which was thrown out on the right—525—became later the main pseudopod. The ameba now moved directly

toward the dark spot until within about twenty microns—528—when the protoplasmic current was reversed and the ameba moved off through a pseudopod on the left near the posterior end—529. Soon this pseudopod was withdrawn and the ameba flowed into another pseudopod (perhaps the vestige of the former main pseudopod) which led the ameba to the right (to the left of the dark spot)—533. After moving some distance in this direction, a pseudopod was thrown out on the left directly opposite to the dark spot—534. Two more pseudopods were then formed on the right through the more posterior of which the ameba moved on —535–537. A pseudopod was then formed on the left which led the ameba out of range of the dark spot—538. The ameba was then shifted with the dark spot on the right and the grain of globulin just in front of it—540. The ameba turned to the left—541—and sent out on the convex side a pseudopod through which the ameba moved forward with the dark spot on the left—543. As the ameba moved on it turned toward the dark beam but presently two pseudopods were thrown out from the middle of the ameba, one on either side—546. (The pseudopod on the left was evidently formed to enable the ameba to move at once into contact with the globulin; the one on the right was formed without assignable cause, although opposite pseudopods are frequently formed under conditions similar to these.) The pseudopod on the left moved through a curved path to the left into contact with the globulin—547–550. When the ameba came into contact with the globulin, it was pushed into the dark area. A slender pseudopod followed it while the tip of the main one remained stationary for the moment, which indicates that the dark area had a deterrent effect on the ameba—551. A food cup was however soon formed and the globulin ingested.

Conclusions.

From these experiments it may be concluded that white light and all the visible spectral colors cause positive responses; but whether all are equally attractive cannot be definitely stated, for experiments would have to be staged differently to produce accurate results. Nevertheless the red end of the spectrum seems to be somewhat more attractive than the blue.

What is of considerable interest in the behavior of ameba toward light is that the character of the response may vary rapidly. See Figs. 22 to 36; 37 to 48; and a number of other experiments. A negative reaction may be followed by a positive and vice versa. There is no definite relationship between ameba and light, on account of which the ameba is always either positive or negative or indifferent. Stimulation from light produces the same general character of reaction as stimulation from glass or carbon. The only observable difference is a quantitative one; light beams are sensed at a greater distance than particles of glass or carbon. This difference may however be due to a difference in intensity of the stimuli.

Ameba reacts to dark spots in much the same way that it does to beams of light. The reactions are either positive, negative or indifferent. But they are negative in much the greater number of cases. But no sooner does one observe the reactions of an ameba to perpendicular beams of light and of darkness than the question arises as to the transfer of the stimulus to the ameba as well as the nature of it. How can an ameba sense a beam of light or darkness which never comes nearer to it than 100 or 150 microns? It is possible that small particles suspended in the water reflect light from a beam of light so as to reach the ameba in much the same way that man can observe a beam of light in a dark room because of the dust particles in the air. But if so the ameba, being eyeless, is wonderfully sensitive to light. But as to beams of darkness the case is entirely different. Is it conceivable that an ameba can sense a beam of darkness at a distance because not as much light is reflected from the particles in the dark beam as from those more brightly illuminated surrounding the beam? If one did not know of reactions to beams of darkness, one might adopt the hypothesis of the reflection of light from particles in the beam; but since similar behavior is observed toward beams of darkness, this explanation is obviously not the right one. Some disturbance is created by the beams which is then radially transmitted; so much is certain. But just what is the nature of the disturbance is not clear.

In a preceding paper (Schaeffer, '16*c*), in which the reactions of ameba to particles of glass, carbon, and similar materials were

described, it was concluded that the nature of the stimulus which enabled amebas to react to these substances at a distance also remains unrecognized. Now it is possible that the nature of the stimulus which makes reaction at a distance possible is the same for all these various test objects, since the reactions are very similar. If so, the nature of the stimulation must be simple and fundamental, such as differences in electrical potential which give rise to electrical currents. But if the nature of the stimulation should be electrical, the quantities of current arising from the various test objects must be infinitesimally small, and very great if not insurmountable difficulties would be encountered in demonstrating the presence of such small currents.

To show the general reactions of ameba to globulin, carbon, etc., when stimulated simultaneously by beams of light or of darkness, the experiments may be classified as follows.

1. Food objects (grains of globulin) were laid *over* a beam of intense light so that the food should be very brightly illuminated —365–372. Blue spectral light was used in the experiments recorded, for blue light has been regarded as more disagreeable than other spectral colors. The globulin was sensed at a distance and the ameba moved toward it and ingested it. There was no definite indication that the blue light had any effect in modifying the behavior unless the pseudopod to the right in Fig. 368 is to be regarded as expressing a deterrent effect of the light. The ameba, in effect, reacted as if no spectral blue light was present.

2. The food substance was laid *some distance from* the green or yellow light, and in various positions with respect to the ameba and the beam of light—373–437.

(*a*) When the green light lay between the ameba and the globulin, the light had a slight disturbing and deterring effect —386. The ameba made a slight detour around the green light. In another test with the experiment similarly staged, the disturbing effect of the green light was more pronounced—373. The ameba made a wide detour around the light and moved into contact with the globulin without coming into contact with the green beam. In both experiments green light, which is positive when sensed alone, became negative in contrast with the more strongly (or differently) positive globulin.

(*b*) In the experiment with yellow light—397–407—the ameba moved straight toward the light after the globulin was within sensing range, then moved over the beam of light, after which the direction of motion was changed so that the ameba moved directly toward the globulin. The globulin was eaten in a typical food cup. The yellow light was not deterrent in this case. But another ameba reacted negatively to both yellow light and globulin, when presented simultaneously, but positively when presented separately. The ameba was satiated or sick, for the globulin was only partially surrounded.

3. Grains of globulin and carbon were laid over beams of darkness.

(*a*) An ameba moved toward a dark spot on which lay a grain of carbon until it came within about thirty microns of the dark spot, when negative behavior set in. The ameba moved away to the right—463. In the succeeding test the ameba reacted at first positively to the dark spot alone, and after that decidedly negatively.

(*b*) A piece of globulin was laid on the dark spot, to the right of the ameba. The ameba moved directly toward the dark spot —globulin—though it seemed to have been slightly deterred by the dark area, for the ameba broke up into two pseudopods—495 —and just when the dark beam was reached a little later, a small side pseudopod appeared. The globulin was however finally ingested. In another experiment the globulin was placed near the far edge of the dark spot—522. The behavior of the ameba became very irregular as it moved near the dark beam. Soon a pseudopod was sent out straight toward the globulin, but it was presently retracted and the ameba moved off to the left, veering to the right. There can be no doubt of the strongly deterrent effect of the dark beams. There can also be no doubt of the strongly attractive effect of the globulin.

Summary.

1. Ameba senses beams of light of twenty microns' diameter that pass no nearer to the ameba than 100 microns or 150 microns. In nearly all cases under these conditions the ameba moves directly toward the beam. When the ameba comes into contact

with the beam it either flows over it indifferently, or it reacts negatively to the beam by changing its direction of movement.

2. Beams of spectral light and of white light have approximately the same general effect. It appears however that spectral light at the blue end is somewhat less attractive than that at the red end.

3. Beams of darkness are also sensed at a distance like beams of light. They are usually negative. That is, the ameba usually avoids the beams before coming very near them.

4. It is the change of light intensity that determines changes in reactions. Neither high nor low intensities seem to be either negative or positive in themselves. Movement from a region of low light intensity into a region of high intensity frequently occurs if the contrast is not too great; but movement toward a region of lower light intensity (dark beams) is seldom seen.

5. No explanation is suggested for the sensing of beams of light and of darkness at a distance. The nature of the stimulus and the means of its transfer in such cases is not known.

6. Grains of globulin illuminated by perpendicular beams of light seem, on the whole, to be at least as attractive as when not more brightly illuminated than the field. But when globulin grains are laid in large dark beams, the ameba frequently shows unmistakable signs of a tendency to react negatively.

7. Both light beams and globulin grains are positive when stimulating the ameba separately; but when a grain of globulin and a beam of light, placed a small distance apart from each other, stimulate the ameba simultaneously, the more weakly positive object—the beam of light—becomes usually strikingly negative.

8. An ameba is positive, negative or indifferent to beams of light depending upon circumstances.

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EXPLANATION OF PLATES.

The figures are camera lucida drawings of sample experiments taken from the laboratory notes without alterations. The camera lucida was attached to the right-hand tube of a long-arm Zeiss binocular microscope, which was used in connection with the stage and condenser of a compound microscope. Eyepieces number 4 and objective a_3 were used, giving a magnification of 65 diameters. A scale by means of which the size of amebas and light beams can be estimated is shown on Plate V.

The figures are numbered serially from 1 on, for reference. The numbers are placed inside the figures. They are to be looked upon as labels only. They have no other significance. An x following a number, as 21x, indicates the end of the experiment illustrated by Figs. 10 to 21x inclusive. A new experiment starts with Fig. 22 and ends with Fig. 36xx, and so on. If a number is followed by xx, it means that the next experiment was performed upon a different ameba. Thus Figs. 1 to 9xx represent the result of a single experiment upon an ameba. With Fig. 10 a new ameba was employed, and so on. The order in which the figures were drawn is represented by the serial numbers for all the figures in any one experiment, and in nearly every case for all the experiments performed on any one ameba.

The time of the beginning and the end of each experiment is given in hours and minutes. In some cases the time of drawing of each figure is also given, and where it is not given it may easily be computed—the figures in such case being spaced equally in time.

The arrows show the direction of active protoplasmic streaming. The larger arrow in the last figure of each experiment denotes the direction the ameba took in moving away from the test object.

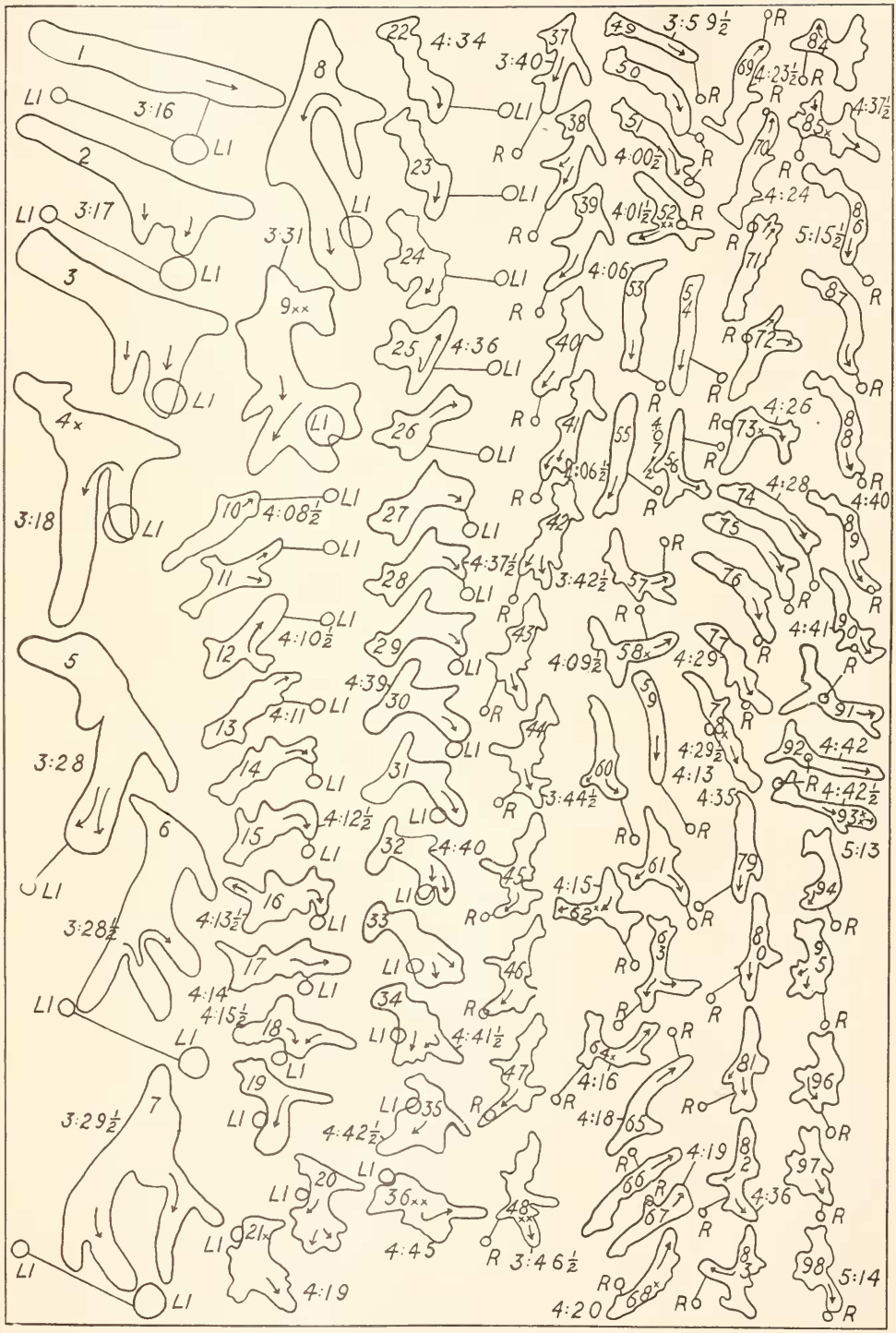
The light beams, etc., are labelled in abbreviated form. See table of abbreviations on below. For quick and correct reference these test objects are connected with the proper ameba by leader lines. These lines have no other significance.

It will be noted that there are slight differences in the size and shape of the same light beam or other test object as drawn in the figures of any single experiment. The explanation for this difference lies in the speed with which the drawings had to be made in order to catch important items of behavior. As a rule the parts of the ameba lying nearest the test object received the most careful attention and were drawn first; the posterior parts of the ameba and the test object were drawn last.

For detailed explanation of figures see pages 49–67 of the text.

Table of Abbreviations.

B, blue light.
CA, carbon.
D, dark beams.
G, globulin.
GN, green light.
LI, white light.
OL, orange light.
R, red light.
V, violet light.
Y, yellow light.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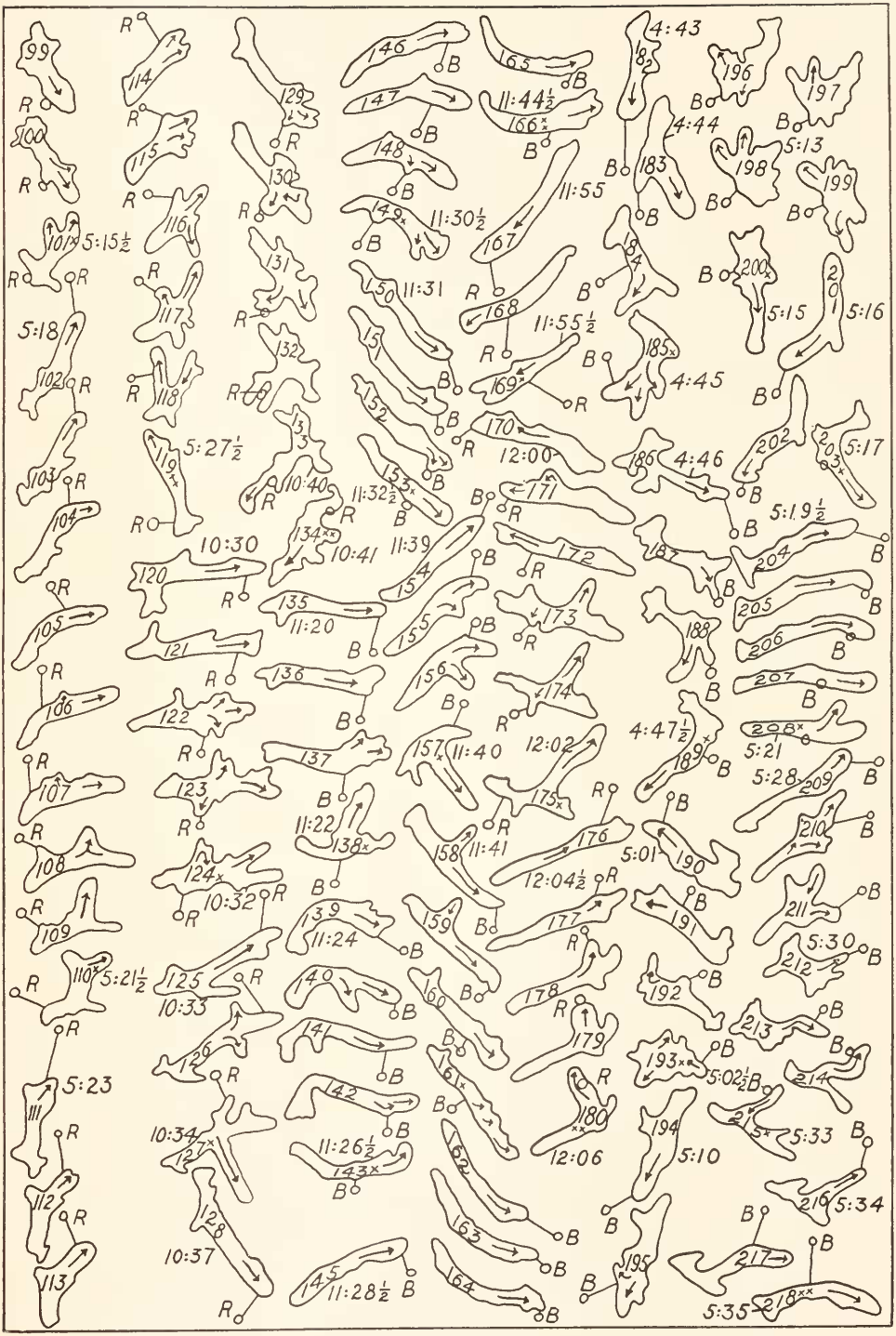
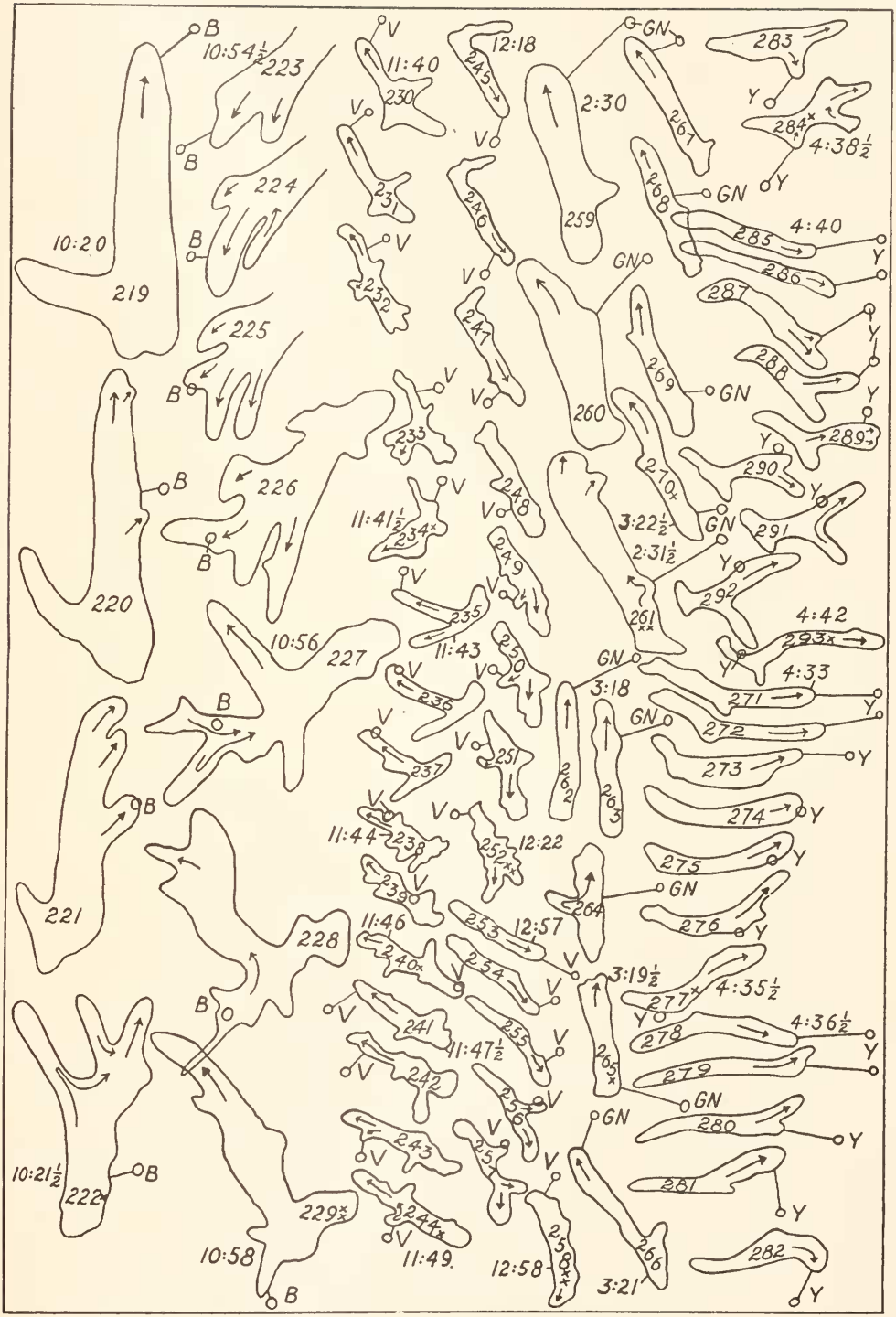


ASA A. SCHAEFFER.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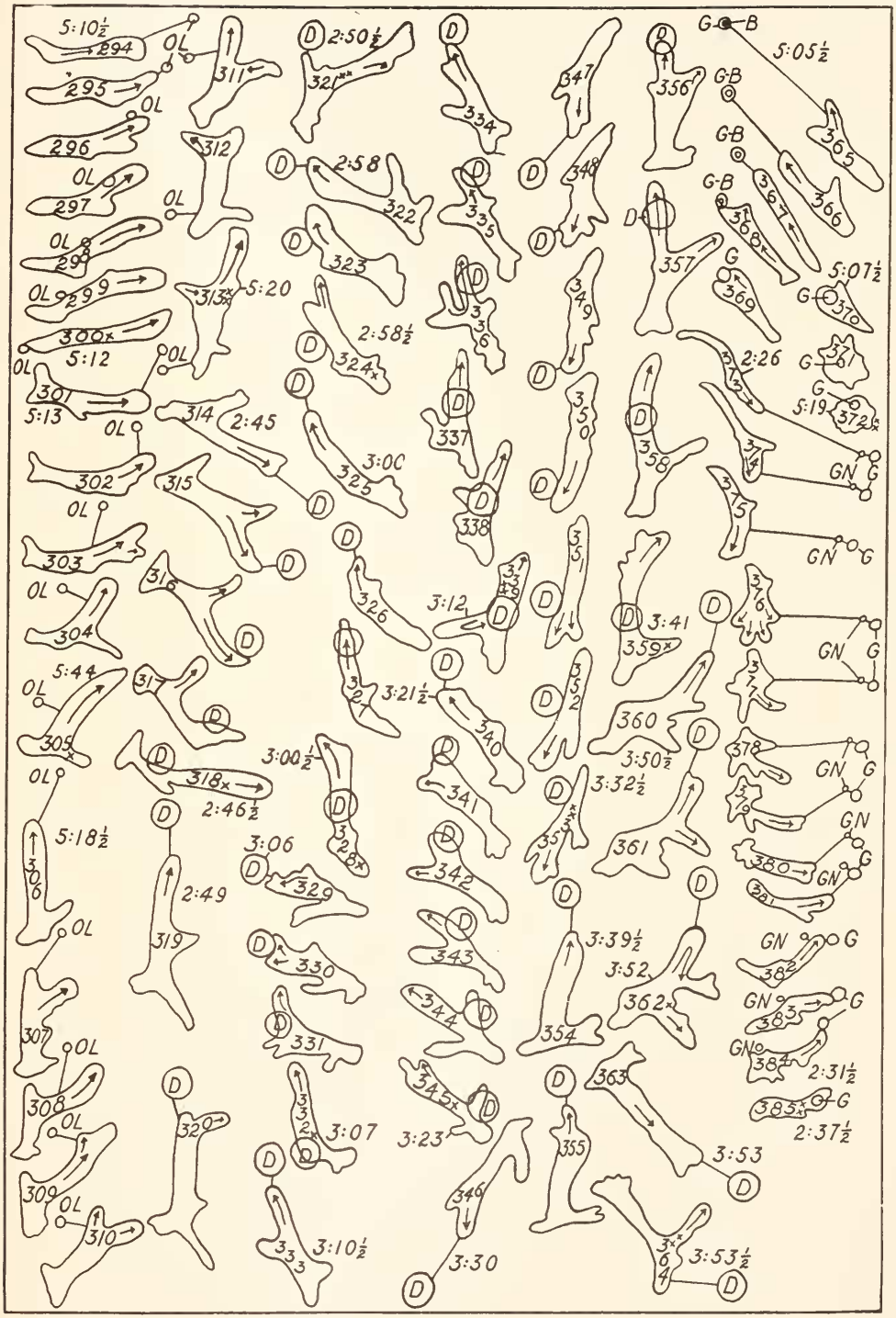
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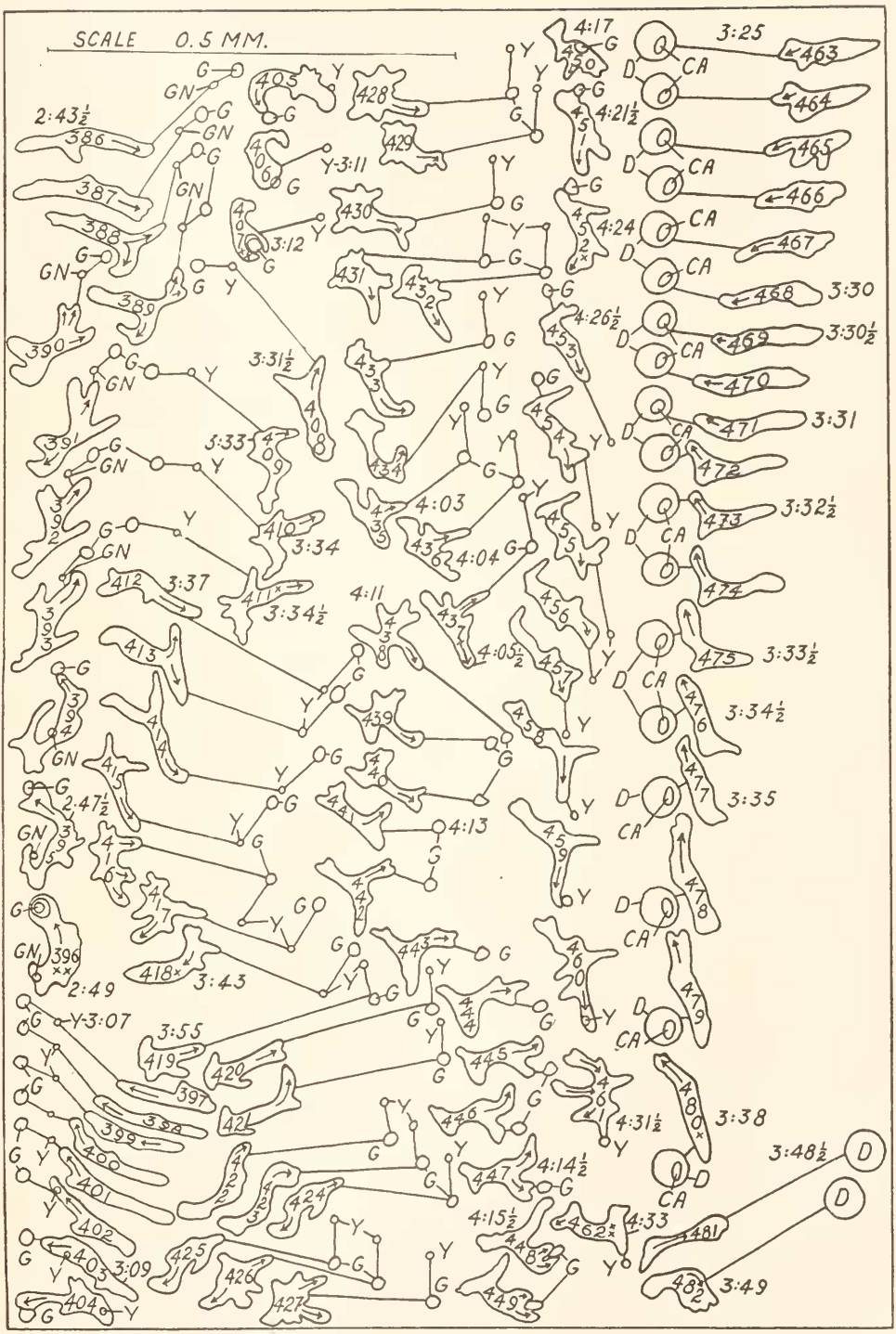
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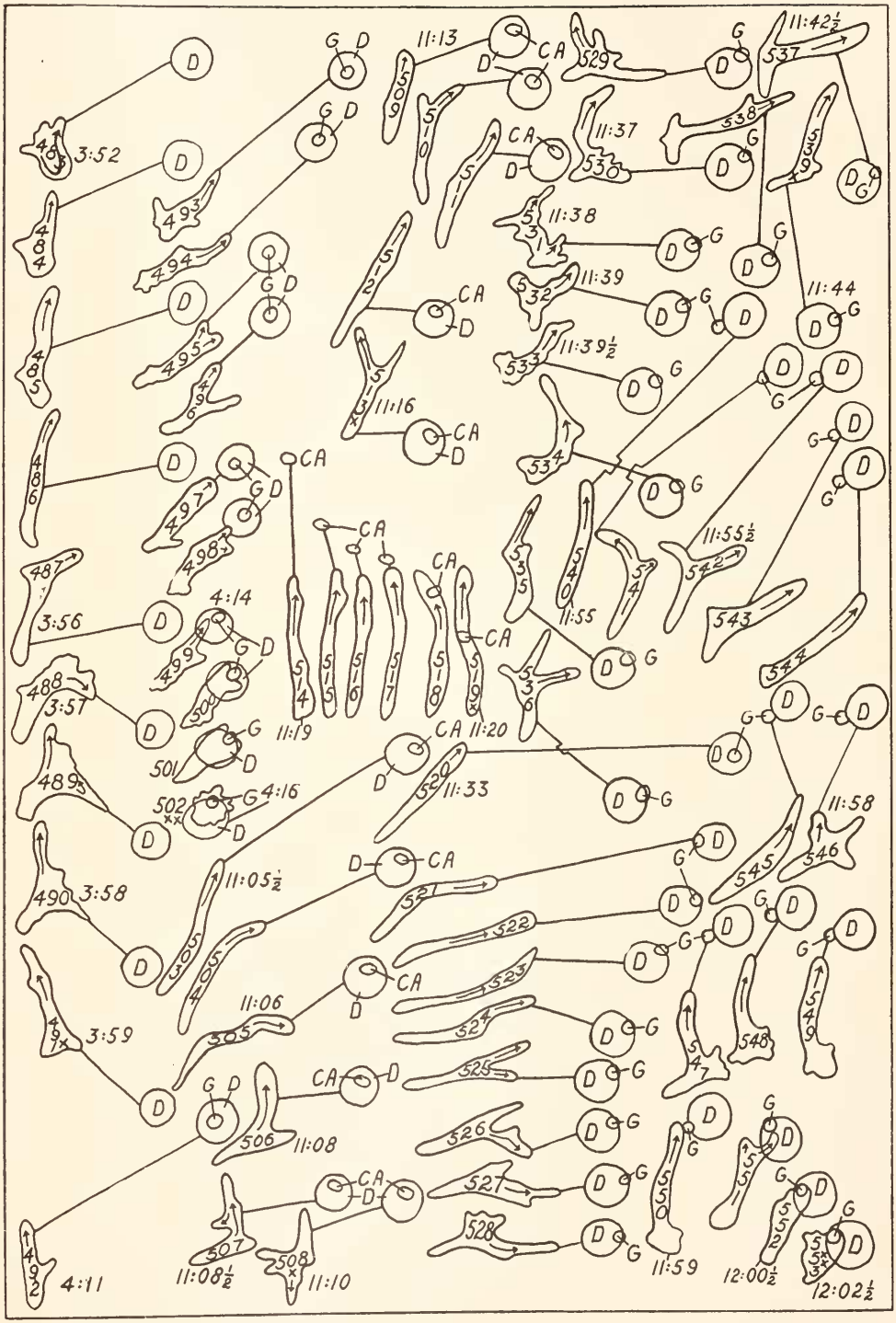
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SUSCEPTIBILITY GRADIENTS IN THE HAIRS OF CERTAIN MARINE ALGÆ.

C. M. CHILD,

WITH FIVE FIGURES.

The colorless hairs or capillary branches so common on the red and the brown algæ are in many cases beautiful objects for the study of the axial susceptibility gradients. A few data obtained during the summer of 1915 at Woods Hole have already been published (Child, '16a). The present paper records the result of further investigation during the summer of 1916 on the physiological polarity of these hairs and includes data on additional species, experimental results on modification of the gradients which answer certain questions raised by the earlier work, and also a correction of the earlier observations concerning one species.

Thus far hairs of the following three types have been examined:

Unbranched unicellular hairs, *Ceramium*, *Chondrus*, *Agardhiella*. The hair is a very slender and delicate outgrowth, sometimes reaching a length of several millimeters, but unicellular.

Unbranched multicellular hairs with basal vegetative tip, *Fucus*, *Castagnea*.

Branched, multicellular hairs with apical vegetative tip, *Chondria*, *Polysiphonia*, *Griffithsia*.

Although this grouping of species according to the form of the hairs does not represent the taxonomic order, it seems the most satisfactory for present purposes since only the hairs are to be considered. In most species examined the hairs are so extremely sensitive that conclusions concerning the existence of a gradient and its direction can be safely drawn only from plants in the best possible physiological condition, collected with the minimum of handling and examined at most within a few hours after collection. Within the first few days in the laboratory, even in running water, the hairs usually die and often drop off, although